Impaired Post-Irradiation Survival of Cyclooxygenase-2-Deficient Mice

MICHAL HOFER[1], ZUZANA HOFEROVÁ[1], ARTIOM GRUZDEV[2], LADISLAV DUŠEK[3], MARTIN FALK[1]

[1]Department of Cell Biology and Radiobiology, Institute of Biophysics, v.v.i., Czech Academy of Sciences, Brno, Czech Republic; [2]Laboratory of Respiratory Biology, Division of Intramural Research, NIEHS/NIH, Research Triangle Park, NC, USA; [3]Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Corresponding author: Michal Hofer, Department of Cell Biology and Radiobiology, Institute of Biophysics of the Czech Academy of Sciences, Královopolská 135, CZ-61265 Brno, Czech Republic. Fax: +420/541211293. E-mail: hofer@ibp.cz.

Short title: Post-irradiation survival of COX-2-deficient mice

Summary

We investigated and evaluated post-irradiation survival in cyclooxygenase-2-deficient (COX-2 KO) mice. Thirty-day survival following exposure of COX-2 KO mice to a lethal dose of 8.5 Gy of γ-rays was observed to be statistically significantly lower in both males and females, as well as when the sexes were merged, in comparisons with their wild-type counterparts. These findings were related to the previous observations concerning the detrimental influence of the COX-2 genetic disruption on hematopoiesis in sublethally irradiated mice. Deteriorated post-irradiation survival of COX-2 KO mice confirmed the previously anticipated conclusion regarding negative influence of the anti-inflammatory action of COX-2 deficiency under the conditions of exposure of the animals to ionizing radiation.

Key words

Cyclooxygenase-2; COX-2 knock-out mice; Ionizing radiation; Post-irradiation survival; Post-irradiation inflammation

Cyclooxygenase-2 (COX-2) enzymatic activity can be limited in animal experiments by two different approaches. First, a pharmacological acute COX-2 inhibition, which is potentially not absolute and may result in partial non-selective co-inhibition of cyclooxygenase-1 (FitzGerald and Patrono 2001, Patrignani and Patrono 2015), can be obtained following administration of various COX-2 inhibitors (e.g., Rayar *et al.* 2017).

Second, effects of a chronic, absolute, and quite selective COX-2 inhibition can be studied using COX-2-deficient (COX-2 KO) mice.

There exists certain controversy concerning the effects of pharmacologically induced COX-2 inhibition on survival of mice exposed to a lethal dose of ionizing radiation. Jiao *et al.* (2009) reported no significant effects of meloxicam, one of the COX-2 inhibitors, on survival of experimental mice and even detrimental effects of the administration of the drug in the conditions of combined radiation injury. On the other hand, in the laboratory of the authors of this communication, administration schemes of meloxicam were developed which provided a statistically significantly higher survival of the meloxicam-treated animals in comparisons with their pharmacologically untreated controls; these schemes comprised timing of meloxicam administration shortly (1 h) either before (Hofer *et al.* 2008) or after irradiation (Hofer *et al.* 2011, 2014).

Taking into account the above mentioned imperfect COX-2 inhibition achieved by pharmacological means, in the present study we used COX-2 KO mice for evaluation of the action of a life-long and total COX-2 inhibition on post-irradiation survival. The findings were also related to previously reported (Hofer *et al.* 2017) findings in hematopoiesis of sublethally irradiated COX-2 KO mice.

Mice

Ten- to 12-week-old male and female COX-2 KO mice and their wild-type (WT) littermates on a true 129B6F1 genetic background were used (Morham *et al.* 1995). The use and treatment of the animals followed the European Community Guidelines. The experiments were approved by the Institute's Ethical Committee.

Irradiation

The mice were whole-body irradiated at a dose rate of 0.31 Gy/min using a γ-ray source (^{60}Co, Chisostat, Chirana, Prague, Czech Republic).

Survival experiments

Survival of the experimental mice was recorded daily up to day 30 after irradiation.

Statistics

Seventeen COX-2 KO males, 27 WT males, 11 KO females, and 43 WT females were used for the survival studies. Analysis of survival time was carried out by Kaplan-Meier methodology. Estimates of mean survival time were derived from the Kaplan-Meier curve. Differences in mean survival time between the individual groups were tested by a log-rank test. Differences in the cumulative 30-day survival between the individual groups were tested by Fisher's exact test. The significance level was set at $P < 0.05$.

As follows from Fig. 1, the post-irradiation survival of the COX-2 KO mice was statistically significantly worse than that of their WT littermates, be it males (Fig. 1A), females (Fig. 1B), or merged sexes (Fig. 1C). In all statistical comparisons, be it mean survival time (log-rank test) or cumulative 30-day survival (Fisher's exact test), the statistical significance was always at the level of $P < 0.001$ (Figs. 1A, 1B, 1C).

Radiation triggers inflammation which manifests itself also in hematopoietic and immune tissues (Hekin *et al.* 2015). COX-2 is an inducible isoenzyme of COX (e.g., Smith *et al.* 2011), responsible for stress-induced synthesis of prostaglandins (e.g., Simmons *et al.* 2004), and, thus, its anti-inflammatory action finds expression, among others, after irradiation

which represents a typical stress factor. The findings of an aggravated post-irradiation survival of COX-2 KO mice, together with the previously observed deep radiation-induced deterioration of hematopoiesis in these animals (Hofer *et al.* 2017), bear evidence about beneficial action of radiation-triggered inflammation that is absent in COX-2-deficient mice.

The lack of COX-2 in the COX-2 KO mice partially mimics chronic administration of COX-2-selective non-steroidal anti-inflammatory drugs (NSAIDs). Mechanisms of damage to the gastrointestinal tract from NSAIDs have been an object of many studies (for review, see Bjarnason *et al.* 2018). The radiation dose of 8.5 Gy used in our survival study can approach the level of the gastrointestinal syndrome of the acute radiation disease (Alpen 1990). Thus, the radiation-induced gastrointestinal damage could combine with that evoked by cyclooxygenase inhibition. Since, however, critical NSAIDs-induced gastrointestinal adverse reactions are mostly dependent on cyclooxygenase-1 inhibition (Suleyman *et al.* 2007), selective COX-2 inhibition in the COX-2 KO mice should not significantly deepen the radiation-induced gastrointestinal damage in these animals and should not significantly contribute to their observed low post-irradiation survival.

Of interests is also the lower number of COX-2 KO offsprings in comparison with the WT ones (the COX-2 KO and WT mice used for the study were born from the same breeding pairs) (see Methods, Statistics). It can follow from this finding that the lifelong lack of COX-2 in the COX-2 KO mice can be also a reason for a higher pre-natal mortality of these animals.

Undoubtedly, further studies are needed for detailed elucidation of the consequencies of the chronic COX-2 deficiency in the COX-2 KO mice.

Conflict of Interests

There is no conflict of interests.

Acknowledgements

This work was supported by the Czech Science Foundation (grant no. P302/12/G157) and the Czech contribution to JINR Dubna (Project of Czech Plenipotentiary and Project 3-Plus-3).

References

ALPEN EL: Radiation Biophysics. Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto, 1990, p. 259.

BJARNASON I, SCARPIGNATO C, HOLMGREN E, OLSZEWSKI M, RAINSFORD KD, LANAS A: Mechanisms of damage to the gastrointestinal tract from nonsteroidal anti-inflammatory drugs. *Gastroenterology* **154**, 500-514, 2018.

FITZGERALD GA, PATRONO C: The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med* **345**: 433-442, 2001.

HEKIN N, CETIN Z, NIKITAKI Z, CORT A, SAYGILI I: Radiation triggering immune response and inflammation. *Cancer Lett* **368**: 156-163, 2015.

HOFER M, HOFEROVÁ Z, DUŠEK L, SOUČEK K, GRUZDEV A: Hematological profile of untreated or ionizing radiation-exposed cyclooxygenase-2-deficient mice. *Physiol Res* **66**, 673-676, 2017.

HOFER M, POSPÍŠIL M, DUŠEK L, HOFEROVÁ Z, KOMŮRKOVÁ D: Agonist of the adenosine A_3 receptor, IB-MECA, and inhibitor of cyclooxygenase-2, meloxicam, given alone or in a combination early after total body irradiation enhance survival of γ-irradiated mice. *Radiat Environ Biophys* **53**: 211-215, 2014.

HOFER M, POSPÍŠIL M, DUŠEK L, HOFEROVÁ Z, WEITEROVÁ L: A single dose of an inhibitor of cyclooxygenase 2, meloxicam, administered shortly after irradiation increases survival of lethally irradiated mice. *Radiat Res* **176**: 269-272, 2011.

HOFER M, POSPÍŠIL M, HOLÁ J, VACEK A, ŠTREITOVÁ D, ZNOJIL V: Inhibition of cyclooxygenase 2 in mice increases production of G-CSF and induces radioprotection. *Radiat Res* **170**: 566-571, 2008.

MORHAM SG, LANGENBACH R, LOFTIN CD, TIANO HF, VOULOUMANOS N, JENNETTE JC, MAHLER JF, KLUCKMAN KD, LEDFORD A, LEE CA, SMITHIES O: Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* **83**: 473-482, 1995.

PATRIGNANI P, PATRONO C: Cyclooxygenase inhibitors: From pharmacology to clinical read-outs. *Biochim Biophys Acta* **1851**: 422-432, 2015

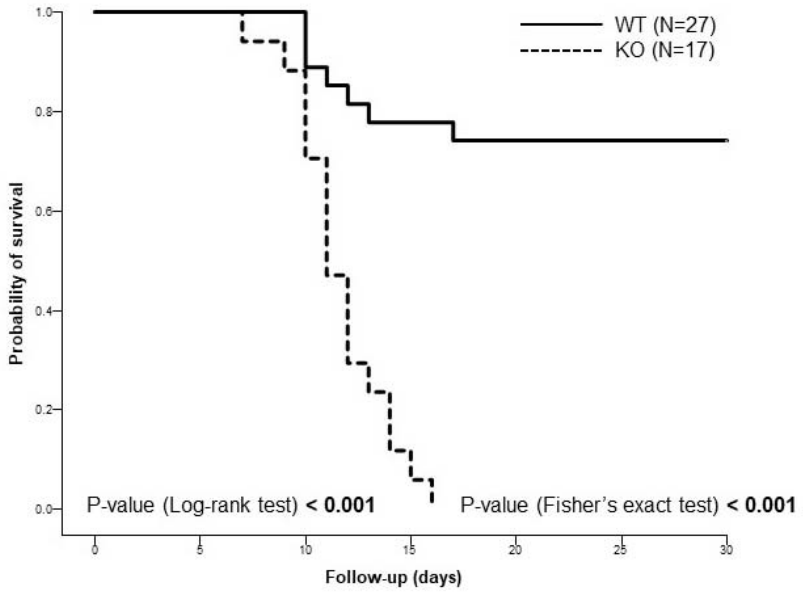
RAYAR AM, LAGARDE N, FERROUD C, ZAGURY JF, MONTES M, VEITIA MS: Update on COX-2 selective inhibitors: Chemical classification, side effects and their use in cancers and neuronal diseases. *Curr Topics Med Chem* **17**: 2935-2956, 2017.

SIMMONS DL, BOTTING RM, HLA T: Cyclooxygenase isoenzymes: The biology of prostaglandin synthesis and inhibition. *Pharmacol Rev* **56**: 387-437, 2004.

SMITH WL, URADE Y, JAKOBSSON P-J: Enzymes of the cyclooxygenase pathway of prostanoid synthesis. *Chem Rev* **111**: 5821-5865, 2011.

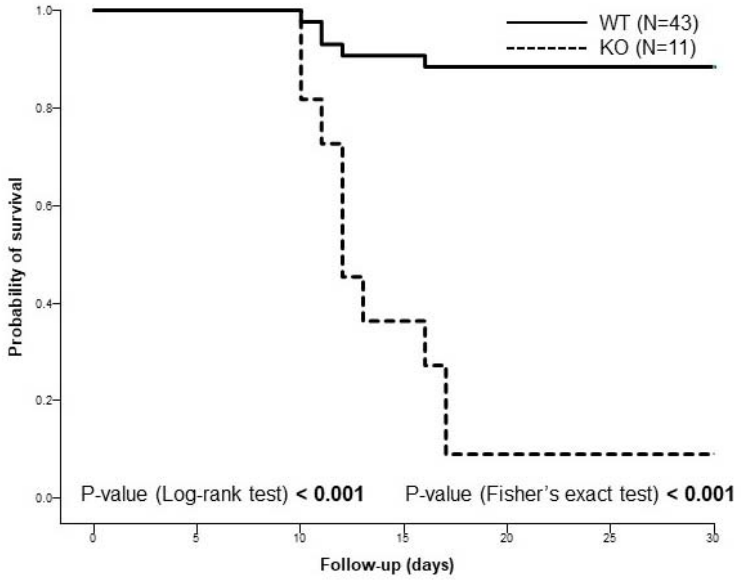
SULEYMAN H, DEMIRCAN B, KARAGOZ Y: Anti-inflammatory and side effects of cyclooxygenase inhibitors. *Pharmacol Rep* **59**, 247-256, 2007.

Fig. 1A. 30-day survival of mice (KO male vs. WT male)



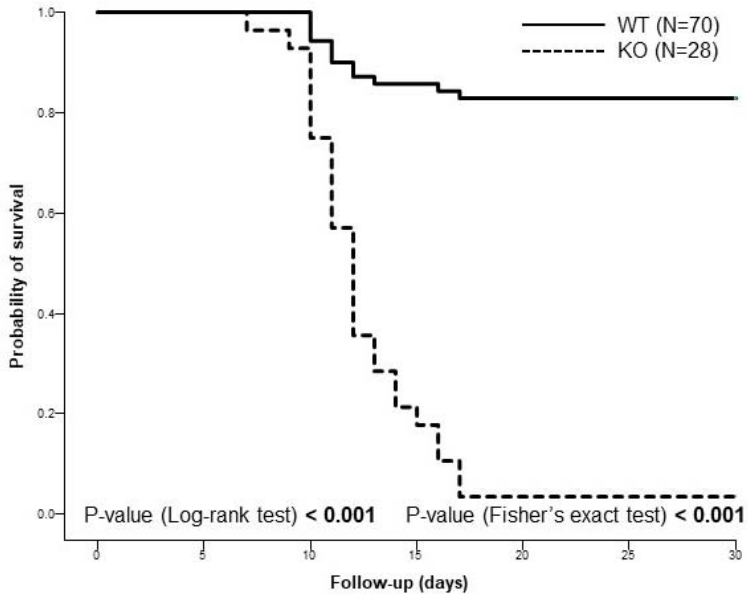
	Number of mice	Number of deaths	Mean survival time (days)
WT	N=27	7 (25.9%)	25.3 (22.3; 28.3)
KO	N=17	17 (100.0%)	11.6 (10.6; 12.7)

Fig. 1B. 30-day survival of mice (KO female vs. WT female)



	Number of mice	Number of deaths	Mean survival time (days)
WT	N=43	5 (11.6%)	27.9 (26.2; 29.6)
KO	N=11	10 (90.9%)	14.5 (11.3; 17.8)

Fig. 1C. 30-day survival of mice (KO vs. WT – merged sex)



	Number of mice	Number of deaths	Mean survival time (days)
WT	N=70	12 (17.1%)	26.9 (25.3; 28.5)
KO	N=28	27 (96.4%)	12.8 (11.3; 14.3)