Relationships Between Some Anesthetics and Nur77 in Pro-B Lymphocytes Apoptosis

OTILIA BOISTEANU[1], GABRIELA ILDIKO ZONDA[2], CATALINA AVRAM[3], LILIANA CHELARU[4]*, CARMEN GENTIMIR[5], DRAGOS ACATRINEI[5], MARCEL COSTULEANU[5]

[1] "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Faculty of Dental Medicine, Department of Anesthesiology, 16, Universitatii Str., 700115, Iasi, Romania

[2] "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Faculty of Medicine, Department of Neonatology, 16, Universitatii Str., 700115, Iasi, Romania

[3] "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Faculty of Medicine, Department of Physiology, 16, Universitatii Str., 700115, Iasi, Romania

[4] "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Faculty of Medicine, Department of Histology, 16, Universitatii Str., 700115, Iasi, Romania

[5] "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Faculty of Dental Medicine, Department of General and Oro-Maxillo-Facial Pathology, 16, Universitatii Str., 700115, Iasi, Romania

There is a total lack of data concerning the relationships between isoflurane anesthetic pharmacologic administration and mitochondrial membrane permeability transition pore opening, induced by orphan receptor Nur77 overactivation, in the apoptosis of the pro-B lymphocytes. The obtained data show that 1 μM Nur77 in the presence of 1% isoflurane for 6 hours induced significant apoptotic effects. Our study thus presented for the first time that Nur77 orphan receptor overactivation might facilitate isoflurane-induced apoptosis in pro-B lymphocytes through the modulation of internal and plasma membrane Ca^{2+} fluxes. Isoflurane by itself is not having such an effect.

Keywords: isoflurane, Nur77, pro-B lymphocyte, apoptosis

The organoprotection induced by inhalational anesthetics is associated with high research interest and has been really demonstrated in many models of organ damage, especially ischemia-reperfusion injury, with emphasis in the perioperative steps and in cardiovascular events [1].

Administered post shoc inhalational anesthetics, beyond surgical settings, might attenuate ischemic injury and confer neuroprotection at subanesthetic doses. Clinically, this would be of increased value in neurological injuries, e.g., stroke, head trauma, and perinatal asphyxia/encephalopathy [1-3].

Isoflurane (a volatile anesthetic) postconditioning is defined as the reduction of ischemic injury after brain ischemia and was demonstrated in rodents (exposed to 2% isoflurane for 60 min immediately after a 90-min middle cerebral arterial occlusion). Thus, isoflurane postconditioning might improve long-term neurological outcome after focal brain ischemia through neuroinflammation fading. The inhibition of NF-kappa B activation and the enhanced production of the proinflammatory cytokine IL-1 beta support the protective effects [4].

On the other hand, neurodegeneration resulting from prolonged exposure to isoflurane in neonatal rats, as well as in neuronal cell cultures, would be reduced through preconditioning (with a short exposure). As demonstrated, the increase of cleaved caspase-3 in the postnatal day 7 rat brain is reduced by preconditioning with a brief isoflurane exposure. There were no obvious observed differences for other known markers of neuronal injury [5].

Isoflurane preconditioning neuroprotective effect is mediated by the pre-activation of the notch signaling pathway. This pathway plays a central role in neural progenitor cell differentiation, as well as in the inflammatory responses underlying central nervous system injury. The inhibition of notch signaling activation dissipated the isoflurane preconditioning-induced neuroprotection. Meanwhile, close results were obtained for notch knockout mice [6].

It is actually demonstrated that exposure during medical residency to waste anesthetic gases in operating rooms increases DNA damage and induces changes in redox status. There is an imperative asking to reduce inhalation anesthetics exposure and to assure better work conditions for physicians [7].

There are important studies suggesting that isoflurane is inducing cell death by disrupting intracellular calcium homeostasis [8-9]. The altered intracellular calcium homeostasis might be related also to mitochondrial malfunction.

Pathologic mechanisms basically related to several degenerative and hyperproliferative conditions (e.g., hypertriglyceridemia, nonalcoholic fatty liver disease, and metabolic syndrome) are centrally including the mitochondria functioning [10].

In many pathologic conditions the oxidative and endoplasmic reticulum stress coexist. It is not clear how alterations in protein-folding environment in the endoplasmic reticulum induce augmentation of oxidative stress. Therefore, the mechanisms which contribute to apoptotic cell death induced by oxidative stress and unfolded protein response (and finally to degenerative diseases) are unknown [11].

Tumoral cells are destroyed by activated photosensitizers through the increased release of reactive oxygen species and triggering of apoptosis mediated by mitochondria [12].

* *email: iliche_histo@yahoo.com*

Improvement of respiratory function in isolated liver mitochondria from mice with murine melanoma might be induced by treatment with betulinic acid, an anti-inflammatory, antiangiogenic, immunomodulatory and anti-tumoral agent. This is done through the enhancement of active respiration (OXPHOS state) [13].

Previous studies demonstrated that isoflurane, as well as sevoflurane, would induce T lymphocytes apoptosis through augmented mitochondrial permeability transition pore opening, release of cytochrome c and caspase-3 activation. These apoptotic mechanisms did not involve the death receptor signaling pathways. Thus, the postoperative competence of human immune system might be compromised during surgery procedures [14].

On the other hand, isoflurane as well as halothane, sevoflurane, and desflurane were able to induce DNA strand breaks (damage) on peripheral B lymphocytes *in vitro* with a concentration-dependent pattern (0.1 mM, 1 mM, 10 mM and 100 mM) [15].

The non-genomic activities of nuclear receptor NR4A1/Nur77/TR3/NGFIB, e.g. the lymphocyte apoptosis-mediated effects, received increased attention in the last two decades research [16].

Since the literature data are lacking, we aimed to study the effects of isoflurane on apoptosis of pro-B lymphocytes, in conjunction with NR4A1/Nur77 orphan receptor activation.

Experimental part

The mouse pro-B cell line Ba/F3 was grown in RPMI 1640 medium (Sigma-Aldrich), supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 10% heat-inactivated FBS (fetal bovine serum), 1 mM Ca^{2+}, and 1 μM recombinant murine IL-3 (Sigma-Aldrich), in an atmosphere with 5% CO_2 and at 37°C, as previously described [17].

The density of Ba/F3 cells before treatment was maintained at 5 x 10^5 per mL. For some experiments (in triplicate), Ba/F3 cells (gift of Dr. Zonda from IFOM-IEO Milan) were treated with 1 μM Nur77 and 1% isoflurane for 6 h. As control we used the effects of Nur77 1 μM, a good inducer of apoptosis through mitochondrial permeability transition pore opening, also in triplicate. An anaesthesia machine was used to deliver 1% isoflurane to a sealed plastic box in a 37°C incubator [9]. The plastic box contained the plates which were seeded with Ba/F3 pro-B cell line. No treatment was applied to the control cells. The mitochondrial membrane potential was watched in the presence of 1 μM JC-1 (Sigma-Aldrich), a very sensitive marker, at 37°C for 30 min.

The laser confocal microscopy was performed using a Microradiance setup (488 and 514 nm), an inverted Nikon Eclipse TE-300 microscope, oil-immersion objective (100X), and LaserSharp software. As emission filter for 488 nm excitation was used the HQ515/530 and HQ530/560 for 514 nm excitation.

The analysis of the collected images (resolution 1280 x 1024) was done using ImageJ, a public domain, Java-based image processing program (National Health Institute, U.S.A.).

Results and discussions

The treatment of Ba/F3 cells with 1 μM Nur77 for 6 hours didn't dissipate significantly the mitochondrial membrane potential (fig. 1) as compared to control cells (fig. 2). The high mitochondrial membrane potential (ψ_{mt}) was associated with almost 90% of the cells in both cases. The high ψ_{mt} is mirrored by bright intensity of red emission

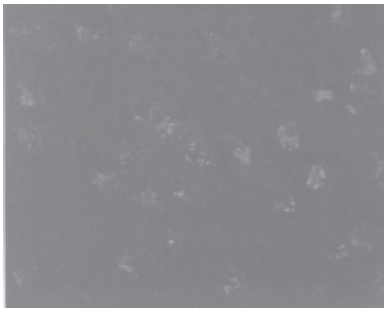

Fig. 1. High mitochondrial membrane potential (ψ_{mt}) is evident by laser confocal microscopy and JC-1 for almost 90% of Ba/F3 cells treated with Nur77 1 μM for 6 h. Representative image of many acquired from three independent experiments (100X)

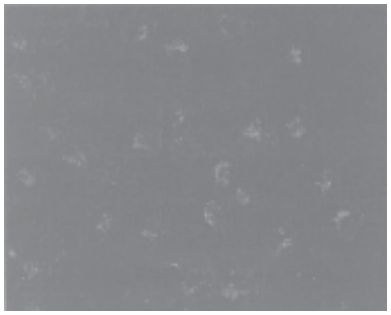

Fig. 2. Ba/F3 control cells, receiving no treatment, show high mitochondrial membrane potential (ψ_{mt}), normal for live cells. Usually, there is a 10% proportion of apoptotic cells all the time, representing their active turnover. Representative image of many acquired from three independent experiments (100X)

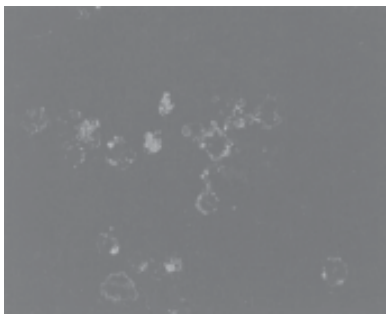

Fig. 3. In the presence of 1 μM Nur77 and 1% isoflurane for 6 hfewer Ba/F3 cells (around 35% on average) associate high mitochondrial membrane potential (ψ_{mt}). There is a large green emission of JC-1, evident for mitochondrial membrane potential dissipation. Representative image of many acquired from three independent experiments (100X).

of JC-1. By contrast, when Ba/F3 cells were treated with Nur77 1 and isoflurane 1% for 6 h, fewer cells (almost 35%) remained alive, with some distinct energized mitochondria (fig. 3).

When pro-B cell line Ba/F3 cells were treated with isoflurane 1% alone for 6 h the dissipation of mitochondrial membrane potential was not observed (personal observation, data not shown). These results suggest that isoflurane is not inducing massive pro-B cells apoptosis when lacking augmented Nur77 orphan nuclear receptor activation. This augmented Nur77 orphan nuclear receptor activation might be necessary for its extranuclear effects.

The well described and induced neuroapoptotic effects of isoflurane were related to the increase of mitochondrial dysfunction and oxidative stress. These ones trigger the opening of mitochondrial permeability transition pores, reactive oxygen species and cytochrome c release, as well as caspase-3 activation [18].

Furthermore, isoflurane activates the IP_3 receptor located on the endoplasmic reticulum membrane, having

as consequences the massive cytosolic calcium release and apoptosis. The isoflurane cytotoxicity is enhanced in the cases of familial Alzheimer or Huntington disease, associating neurons' vulnerability through IP_3 receptor altered functioning [19].

Isoflurane might have as direct molecular target the IP_3 receptor. But it is not clear this moment if the anesthetic effects are directly due to the modulation of intracellular calcium channels. That includes also the membrane endoplasmic reticulum calcium channels, overactivated by isoflurane in clinical conditions [20].

As a paradoxical finding, moderate increases in intracellular calcium could be neuroprotective. In rat cortical slices, isoflurane treatment reduces the calcium influx mediated by glutamate receptors, initially stimulated by NMDA, L-glutamate, or ischemia [1, 21]. In these conditions, higher concentrations of cytosolic ionic calcium might be equivalent with neuroprotection.

Isoflurane, sevoflurane, as well as propofol, all interact with the leukocyte adhesion molecule leukocyte function-associated antigen-1 (LFA-1). LFA-1 is a critical adhesion molecule for leukocyte arrest. The measured result of such interaction is represented by the consecutive inhibition of the interleukin-2 (IL-2) production and leukocyte functioning alteration. All the above anesthetics share the lovastatin binding site in LFA-1 [22].

The capacity of isoflurane to trigger oxidative DNA damage is a little known. Anyway, surgery is obviously associated with a transient alteration of perioperative and postoperative immunological status. Lymphocytopenia represents a common phenomenon. It was found that DNA repair and apoptosis-related genes might be clear down-regulated on the first post-operative day. Isoflurane was not inducing genotoxicity and cytotoxicity in lymphocytes, and the toxicogenomic effect in leukocytes was lacking [23].

Conclusions

Our study clearly showed that overactivation of Nurr77 orphan receptor might facilitate isoflurane-induced apoptosis in pro-B lymphocytes of type Ba/F3 through the modulation of internal and plasma membrane Ca^{2+} fluxes.

Acknowledgements: Ph.D. Student Dr. Acatrinei D. is the recipient of a grant CERO-Profil de Carieră: Cercetător Român, POSDRU/159/1.5/S/135760, achieved at the University of Medicine and Pharmacy "Grigore T. Popa" - Iaşi.

References

1.WU, L., ZHAO, H., WANG, T., PAC-SOO, C., MA, D., J. Anesth., **28**, 2014, p. 740.
2.KITANO, H., JEFFREY, R.K., PATRICIA, D.H., STEPHANIE, J.M., J. Cereb. Blood Flow Metab., **27**, 2007, p. 1108.
3.WANG, L., TRAYSTMAN, R.J., MURPHY, S.J., Curr. Opin. Pharmacol., 8, 2008, p. 104.
4.LI, H., YIN, J.B., LI, L.L., DENG, J., FENG, C.Z., ZUO, Z.Y., Neurobiol. Dis., **54**, 2014, p. 216.
5.PENG, J., DROBISH, J.K., LIANG, G., WU, Z., LIU, C.X., JOSEPH, D.J., ABDOU, H., ECKENHOFF, M.F., WEI, H.F., Anesth. Analg., **119**, 2014, p. 939.
6.ZHANG, H.P., SUN, Y.Y., CHEN, X.M., YUAN, L.B., SU, B.X., MA, R., ZHAO, R.N., DONG, H.L., XIONG, L.Z., Neuromol. Med., **16**, 2014, p. 191.
7.PAES, E.R.D., BRAZ, M.G., de LIMA, J.T., da SILVA M.R.G., de SOUSA, L.B., LIMA, E.S., de VASCONCELLA, M.C., BRAZ, J.R.C., Acta Cir. Bras., **29**, 2014, p. 280.
8.YANG, H., LIANG, G., HAWKINS, B.J., MADESH, M., PIERWOLA, A., WEI, H., Anesthesiology, **109**, 2008, p. 243.
9.WANG, H., DONG, Y., ZHANG, J., XU, Z., WANG, G., SWAIN, C.A., ZHANG, Y., XIE, Z., Brit. J. Anaesth., **113**, 2014, p. 695.
10.KERTI, V., CORICOVAC, D., DUICU, O., DANILA, M., DEHELEAN, C., FAGADAR-COSMA, E., MUNTEAN, D., NOVEANU, L., Rev. Chim. (Bucharest), **65**, no. 4, 2014, p. 447.
11.CAO, S.S., KAUFMAN, R.J., Antioxid. Redox Signal, **21**, 2014, p. 396.
12.TAMPA, M., MATEI, C., POPESCU, S., GEORGESCU, S.R., NEAGU, M., CONSTANTIN, C., ION, R.M., Rev. Chim. (Bucharest), **64**,no.6, 2013, p. 639.
13.DUICU, O., CIURLEA, S., DEHELEAN, C., ARDELEAN, S., ANDRICA, F., MUNTEAN, D., SOICA, C., ANTAL, D., CORICOVAC, D., PINZARU, I., Rev. Chim. (Bucharest), **65**, no. 8, 2014, p. 956.
14.LOOP, T., DOVI-AKUE, D., FRICK, M., ROESSLEIN, M., EGGER, L., HUMAR, M., HOETZEL, A., SCHMIDT, R., BORNER, C., PAHL, H.L., GEIGER, K.K., PANNEN, B.H., Anesthesiology, **102**, 2005, p. 1147.
15.KAYMAK, C., KADIOGLU, E., COSKUN, E., BASAR, H., BASAR, M., HUM. EXP. TOXICOL., **12**, 2012, p. 1207.
16.PAWLAK, A., STRZADALA, L., KALAS, W., Steroids, **95**, 2015, p. 1.
17.ZONDA, G.I., GORIUC, A., IANCU, R.I., COSTULEANU, M., ANAL. ŞT. UNIV. Al. I CUZA IAŞI. SECT. GEN. BIOL. MOLEC., **TOM XI**, 2010, p. 15.
18.BAI, T., DONG, D.S., PEI, L., Int. J. Mol. Med., **32**, 2013, p. 819.
19.WEI, H., LIANG, G., YANG, H., WANG, O., HAWKINS, B., MADESH, M., WANG, S., ECKENHOFF, R.G., Anesthesiology, **108**, 2008, p. 251.
20.JOSEPH, J.D., PENG, Y., MAK, D.O.D., CHEUNG, K.H., VAIS, H., FOSKETT, J.K., WEI, H.F., Anesthesiology, **121**, 2014, p. 528.
21.BICKLER P.E., BUCK L.T., HANSEN B.M., Anesthesiology, **81**, 1994, pp. 1461.
22.YUKI, K., BU, W., XI, J., SHIMAOKA, M., ECKENHOFF, R., Anesth. Analg., **117**, 2013, p. 803.
23.BRAZ, M.G., MAZOTI, M.A., GIACOBINO, J., BRAZ, L.G., GOLIM, Mde A., FERRASI, A.C., de CARVALHO, L.R., BRAZ, J.R., SALVADORI, D.M., Mutagenesis, **26**, 2011, p. 415

Manuscript received: 18.12.2014