Melatonin as a Protective Agent *In Utero* : Role in Foetal Programming

Cathy Vaillancourt and Lucas Sagrillo-Fagundes

INRS-Institut Armand-Frappier and Center for Interdisciplinary Research on Well-Being, Health, Society and Environment, Université du Québec, QC, Canada.

cathy.vaillancourt@iaf.inrs.ca

Melatonin mediates, through receptor dependent and receptor independent paths, the survival of normal cells and the death of cancer cells. There is also evidence that melatonin may offer protection against cancer, potentially through the modulation of a wide range of molecular mechanisms: including autophagy; mitochondrial viability, endoplasmic reticulum (ER)-stress and apoptosis. Melatonin has a wide range of functions, but may be particularly important as a free radical scavenger, contributing to protection against oxidative stress. It is an evolutionarily ancient, well-conserved molecule, with powerful antioxidant properties. Notably, its scavenging activities generate new products that themselves have antioxidant properties, creating a cascade whereby each melatonin molecule can ultimately quench up to ten free radicals.

The first 1000 days of life, including time spent in the womb is critical to both immediate and long-term health, and placental function is vital for much of this period. The placenta plays a key role in protecting the foetus from noxious insults that might for example initiate cancer development. The placenta's structure ensures that, although foetal and maternal circulation do not mix, there is a large surface area through which materials can diffuse from one circulation to another. The primary barrier function is the trophoblast layer, which consists of two layers of cells – a layer of stem-cell-like the villous cytotrophoblasts, which act as a source of new cells that fuse to the syncytiotrophoblast, to create a single giant syncytial cell. All material entering the foetal circulation from the maternal circulation (and vice versa) must cross the syncytiotrophoblast. However, it is not a total barrier, and is also turning out to have its own biological functions that could influence the risk of diseases such as cancer. Perhaps less well appreciated is the fact that the placenta is also a site of melatonin production and express its receptors MT1 and MT2 (Iwasaki et al., 2005, Lanoix et al., 2008, Soliman et al., 2015). One of melatonin's most important roles may be in maintaining placental function and protecting the health of the foetus *in utero*. The homoeostasis of the placenta is carefully regulated, but can be affected by oxidative stress, leading to enhanced cell turnover and compromised placental function. This increases the risk of intrauterine growth restriction, pre-eclampsia and preterm birth. Given its protective properties, our group suggested that melatonin might have a role in preventing oxidative damage across the pregnancy. Indeed, circulating levels of melatonin is significantly higher during pregnancy, peaking at birth, and returning to non-pregnant woman levels after delivery (Kivela, 1991). In addition, serum and placental melatonin levels were markedly reduced in both pre-eclampsia and foetal growth restriction. (Lanoix et al., 2012, Nakamura et al., 2001).

To explore further the role of melatonin in normal and abnormal pregnancies, we established an *in vitro* model based on primary villous trophoblast cells culture under normoxia and hypoxia/reoxygenation (Sagrillo-Fagundes et al., 2018). Hypoxic insult to these cells triggered the aberrant activation of oxidative stress and inflammatory mediators, which led to increased apoptosis. These responses were substantially mitigated when exogenous melatonin was added to the culture, throughout the regulation of inflammation and improvement of the autophagic protective activity arguing for an important protective role for this indolamine. Very recently, melatonin has been shown to have efficacy in the management of pre-eclampsia, with melatonin decreasing levels of prematurity and lowering the need for antihypertensive treatment (Hobson et al., 2018)

Interestingly, our group has shown that melatonin induces intrinsic apoptosis in tumour (choriocarcinoma) and promotes survival in non-tumour trophoblast (primary villous) cells, making it a 'smart' killer (Lanoix et al.2012). Recent data of our group indicates that melatonin disrupts the autophagic turnover and the activation of the protective transcription factor Nrf2 to trigger the

disruption of the choriocarcinoma homeostasis (Sagrillo-Fagundes, *unpublished data*). Moreover, melatonin leads to the induction of ER-stress and consequently to the death of placental choriocarcinoma cells (Bienvenue-Pariseault, *unpublished data*). This dual effect of melatonin on apoptosis in tumour versus normal trophoblast cells demonstrates that the trophoblast is a good model to study the different effect of melatonin on normal vs. cancer cells.

There is also growing evidence that maternal melatonin can influence foetal circadian rhythms, by influencing the foetal suprachiasmatic nucleus directly or indirectly through its effects on maternal rhythms. A main issue involving melatonin and the foetal development is the fact that foetus and new-born until three months are not able to produce melatonin, having all its supply via umbilical cord and then via breastfeeding (Anderson et al., 2017). So, if the maternal circadian and placental production of melatonin is impaired, it can lead to lower levels during the foetal development, with long-term issues. In this case melatonin acts mainly as a protective molecule, able to recover the optimal neuronal development in cases of lack of oxygenation in the foetal brain. The lack of oxygenation is fairly common in intrauterine growth restriction and preterm birth, and melatonin treatment has been shown to reduce the extension of its effects (Carloni et al., 2008). To date, no major adverse effect has been described in children and adults after chronic or acute treatments with melatonin. Furthermore, exogenous melatonin does not suppress endogenous melatonin secretion (Sagrillo-Fagundes et al., 2018). Melatonin clinical trials to protect preterm new-born are currently being conducted. However the length of the dose of the treatment still should be optimized to generate reliable results (El-Gendy et al., 2018).

This talk will present the action of melatonin in placental function and discuss the potential protective roles of this indolamine in compromised pregnancy and foetal programming, focusing on its involvement in redox mechanisms. I will try to convince you that the human placental trophoblastic cell provides a unique approach to discover the mechanisms by which melatonin play a pro-survival and cytoprotective role crucial for foetal development and programming. Collectively, this talk will present the protective role of melatonin in trophoblast cells, its action in normal and abnormal (such as choriocarcinoma) cellular processes, and its potential role for the treatment of a variety of disorders, including obstetric complications and cancer.

Selected references

Anderson*, et al.* 2017. Biomol Concepts*,* 8**,** 185-195.
Carloni*, et al.* 2008. J Pineal Res*,* 44**,** 157-64.
El-Gendy*, et al.* 2018. J Matern Fetal Neonatal Med*,* 31**,** 2299-2303.
Hobson*, et al.* 2018. J Pineal Res**,** e12508.
Hobson*, et al.* 2013. BMJ Open*,* 3**,** e003788.
Iwasaki*, et al.* 2005. J Pineal Res*,* 39**,** 261-5.
Kivela 1991. Acta Endocrinol (Copenh)*,* 124**,** 233-7.
Lanoix*, et al.* 2008. J Pineal Res*,* 45**,** 50-60.
Lanoix*, et al.* 2012. J Pineal Res*,* 53**,** 417-25.
Lanoix*, et al.* 2013. Mol Cell Endocrinol*,* 381**,** 35-45.
Miller*, et al.* 2014. J Pineal Res*,* 56**,** 283-94.
Nakamura*, et al.* 2001. J Pineal Res*,* 30**,** 29-33.
Sagrillo-Fagundes*, et al.* 2018. Methods Mol Biol*,* 1710**,** 277-283.
Sagrillo-Fagundes*, et al.* 2018. J Pineal Res (under review)
Soliman*, et al.* 2015. J Pineal Res*,* 59**,** 38-46.