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Trichostatin A induces cell death at the concentration recommended to differentiate the RGC-5 cell line

Sven Schnichels *, Maximilian Schultheiß, Johanna Hofmann, Peter Szurman, Karl Ulrich Bartz-Schmidt, Martin S. Spitzer

Centre for Ophthalmology, University Eye Hospital Tübingen, Schleichstr. 12/1, D-72076 Tübingen, Germany

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ABSTRACT

Supplementation with Trichostatin A (TSA) has been described as the method of choice for differentiating the RGC-5 cell line into cells with neuronal properties. However, TSA is known to induce apoptosis. We therefore investigated whether TSA at the recommended concentration for differentiation (500 nM) and at three additional concentrations (40, 150 and 2000 nM) induces apoptosis or cell death in the RGC-5 cell line.

Morphological changes of the RGC-5 cells occurred after 24 and 48 hours (h) of treatment with 500 and 2000 nM TSA. Differentiation of RGC-5 cell began at 150 nM. A decrease in the cell count was observed from 150 nM TSA onwards compared to controls. Five hundred nanomolar of TSA reduced the amount of cells to 51% ($p < 0.005$) after 24 h and to 24% ($p < 0.005$) after 48 h compared to controls on crystal violet staining. At 500 nM TSA a massive induction of apoptosis after 24 and 48 h was noted. Supplementation of 500 nM TSA increased caspase 3/7 activity 5.0-fold ($p < 0.005$). Furthermore, 27× more TUNEL-positive cells were found and the cleaved caspase 3/caspase 3 ratio was 1.8-fold ($p < 0.1$) higher 24 h after the addition of 500 nM TSA. The Bax/Bcl-2 ratio was 3.4-fold ($p < 0.05$) higher after 48 h. Cell viability decreased to 70% ($p < 0.005$) and to 35% ($p < 0.005$) after 24 and 48 h, respectively. Moreover, 103× ($p < 0.05$) more dead cells (via propidium iodide staining) were found after 48 h of treatment with 500 nM TSA.

In conclusion, TSA induces cell death and apoptosis at the concentration recommended for differentiation. The induction of apoptosis occurred dose and time dependently and already at even lower concentrations of TSA which did not lead to differentiation induced apoptosis. Thus, studies with RGC-5 cells should not be performed within the first 48 h after supplementation with TSA.

1. Introduction

In 2001, a cell line – RGC-5 – was established by the transformation of postnatal day 1 retinal cells with the psi2 E1A virus (Krishnamoorthy et al., 2001). Initially, RGC-5 cells had characteristics of retinal ganglion cells or neurons in general (Krishnamoorthy et al., 2001). They expressed several neuronal markers and were sensitive to neurotrophin withdrawal, serum deprivation and oxidative stress (Krishnamoorthy et al., 2001; Charles et al., 2005; Maher and Hanneken, 2005a,b). However, they also showed features which were not characteristic for RGCs or postnatal neurons. They were mitotically active, though less sensitive to glutamate than primary neurons and looked more like glial cells in cell culture (Krishnamoorthy et al., 2001), although they are not (Krishnamoorthy et al., 2001; Wood et al., 2010). Nevertheless, RGC-5 cells were and are widely used in eye and especially ganglion cell research. Up to now, there are over 150 papers listed in PubMed, which deal with RGC-5 cells in the context of RGC or neuronal research.

However, over the years, the characteristics of the RGC-5 cells have changed. They no longer express certain RGC-typical markers like neurofilaments or the Thy 1.2 receptor or only at very low level, and have become less sensitive to glutamate excitotoxicity (Aoun et al., 2003; Kumar et al., 2005; Maher and Hanneken, 2005b; Fan et al., 2006; Harper et al., 2009; Van Bergen et al., 2009; Ganapathy et al., 2010; Wood et al., 2010). Furthermore, they also expressed mouse specific genes (Van Bergen et al., 2009). Additionally, it is at the moment unclear if the worldwide distributed RGC-5 cells are of rat RGC origin or other retinal cells (Van Bergen et al., 2009; Wood et al., 2010).

Abbreviations: PI, propidium iodide; RGC, retinal ganglion cell; h, hours; NF, neurofilament; n.s., not significant; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

* Corresponding author. Tel.: +49 70712984015; fax: +49 7071294935.
E-mail address: sven.schnichels@med.uni-tuebingen.de (S. Schnichels).

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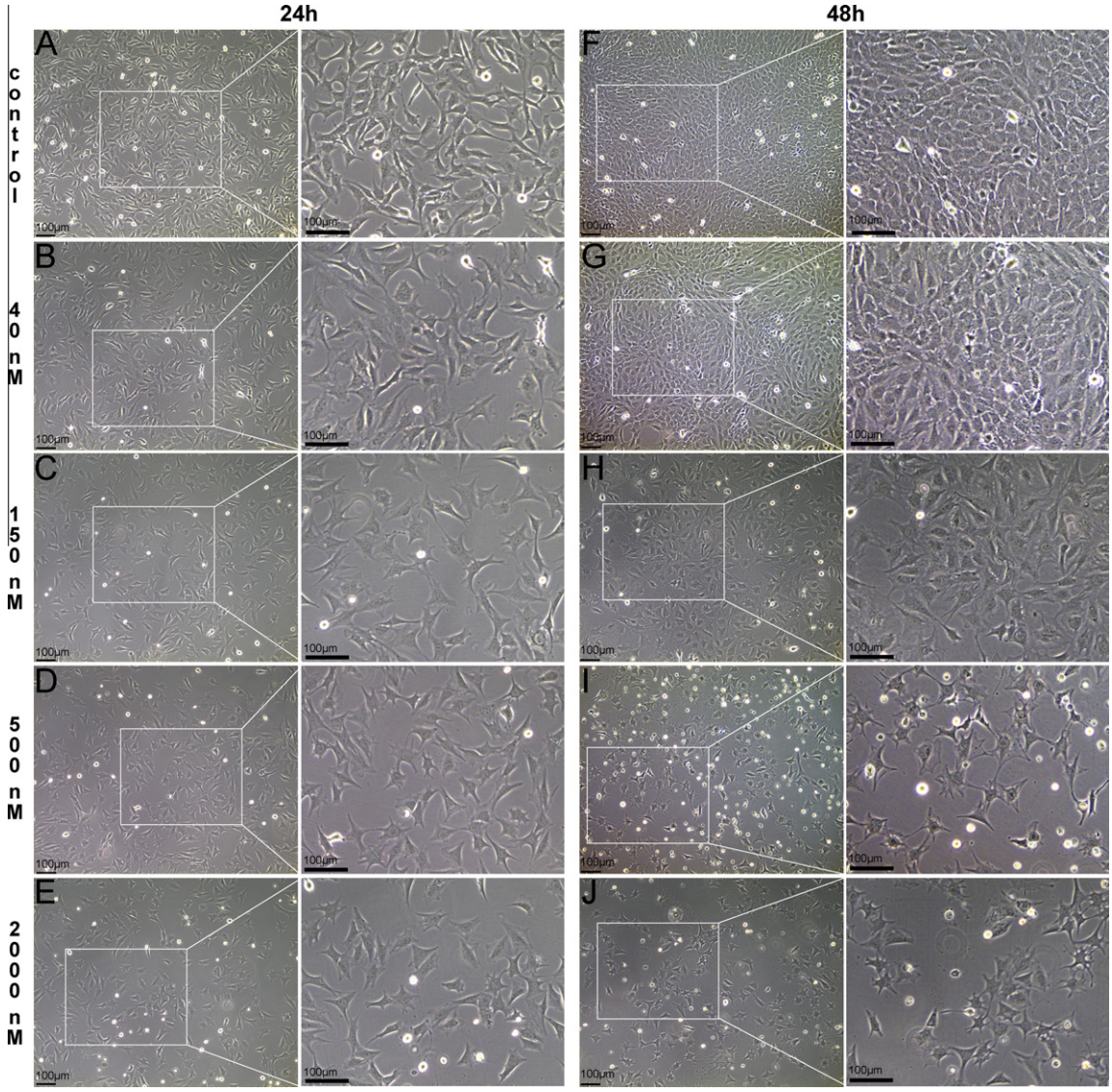

Fig. 1. TSA induces cell morphology changes in RGC-5 cells. Representative phase contrast pictures of RGC-5 cells 24 and 48 h after supplementation with (A/F) control, (B/G) 40 nM, (C/H) 150 nM, (D/I) 500 nM (E/J) 2000 nM of TSA ($n = 4$). Morphological changes of the RGC-5 cells were observed by phase contrast pictures 24 h after treatment with concentrations of 500 and 2000 nM TSA. However, very sporadically single cells also changed their morphology at concentrations of 150 nM. Forty-eight hours after treatment most cells at 500 nM and all cells at 2000 nM TSA concentration showed altered morphology. Many cells at 150 nM TSA were unchanged. Obviously fewer cells were observable at concentrations of 40 nM (only 48 h), 150 nM (only 48 h), 500 and 2000 nM compared to controls ($p < 0.05$).

To preserve this, the only available RGC or eye-neuronal-cell-line, efforts were undertaken to differentiate the RGC-5 cells into cells which share more characteristics with primary RGCs or neurons. The best results were reported using staurosporine (Frassetto et al., 2006; Harvey and Chintala, 2007; Lieven et al., 2007), a non-selective protein kinase inhibitor, and trichostatin A (TSA), a histone deacetylase inhibitor (Schwechter et al., 2007; Wood et al., 2010). Using these substances, RGC-5 cells showed more neuronal or ganglion cell-like morphology and expressed typical RGC and neuronal markers (Frassetto et al., 2006; Lieven et al., 2007; Schwechter et al., 2007; Harper et al., 2009; Ganapathy et al., 2010; Wood et al., 2010). The optimal concentration for the differentiation of RGC-5 cells was determined at 316 nM for staurosporine and 500 nM for TSA, respectively (Frassetto et al., 2006; Schwechter et al., 2007; Wood et al., 2010). Since 2006, staurosporine especially has become the agent of choice to differentiate RGC-5 cells (Harvey and Chintala, 2007; Lieven et al., 2007; Rock and Chintala, 2008; Harper et al., 2009; Lin et al., 2009; Ganapathy et al., 2010). At this point the origin of the cells makes no difference as they can be turned into cells with neuronal and RGC properties with both substances and used for studies on neuronal diseases of the retina, like glaucoma.

However, for both substances these concentrations are known to induce apoptosis in primary neuronal cells or the developing retina (Wallace et al., 2006; Chen and Cepko, 2007; Schallenberg et al., 2009; Gaub et al., 2010; Biermann et al., 2011). Recently, we and others reported that staurosporine also induces apoptosis in every concentration that leads to differentiation and even more, in concentrations that do not lead to differentiation (Ganapathy et al., 2010; Nieto et al., 2010; Schultheiss et al., 2012).

The aim of this study was to investigate whether TSA at the recommended concentrations induces apoptosis or cell death in RGC-5 cells and if another concentration might induce differentiation without the induction of apoptosis or cell death. Therefore, three additional concentrations were tested.