Toxicology and Applied Pharmacology 311 (2016) 61–73

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Effects of *Trans*-Resveratrol on hyperglycemia-induced abnormal spermatogenesis, DNA damage and alterations in poly (ADP-ribose) polymerase signaling in rat testis

Ala` Abdelali [a], Maie Al-Bader [b], Narayana Kilarkaje [a,*]

[a] Department of Anatomy, Faculty of Medicine, Kuwait University
[b] Department of Physiology, Faculty of Medicine, Kuwait University

ARTICLE INFO

Article history:
Received 21 July 2016
Revised 10 September 2016
Accepted 24 September 2016
Available online 27 September 2016

Keywords:
Cell survival
DNA damage
Germ cell toxicity
Glucotoxicity
Oxidative stress
Parthanatos
Type 1 diabetes mellitus

ABSTRACT

Diabetes induces oxidative stress, DNA damage and alters several intracellular signaling pathways in organ systems. This study investigated modulatory effects of *Trans*-Resveratrol on type 1 diabetes mellitus (T1DM)-induced abnormal spermatogenesis, DNA damage and alterations in poly (ADP-ribose) polymerase (PARP) signaling in rat testis. *Trans*-Resveratrol administration (5mg/kg/day, ip) to Streptozotocin-induced T1DM adult male Wistar rats from day 22–42 resulted in recovery of induced oxidative stress, abnormal spermatogenesis and inhibited DNA synthesis, and led to mitigation of 8-hydroxy-2'-deoxyguanosine formation in the testis and spermatozoa, and DNA double-strand breaks in the testis. *Trans*-Resveratrol aggravated T1DM-induced up-regulation of aminoacyl tRNA synthetase complex-interacting multifunctional protein 2 expression; however, it did not modify the up-regulated total PARP and down-regulated PARP1 expressions, but recovered the decreased SirT1 (Sirtuin 1) levels in T1DM rat testis. *Trans*-Resveratrol, when given alone, reduced the poly (ADP-ribosyl)ation (pADPr) process in the testis due to an increase in PAR glycohydrolase activity, but when given to T1DM rats it did not affect the pADPr levels. T1DM with or without *Trans*-Resveratrol did not induce nuclear translocation of apoptosis-inducing factor and the formation of 50 kb DNA breaks, suggesting to the lack of caspase-3-independent cell death called parthanatos. T1DM with or without *Trans*-Resveratrol did not increase necrotic cell death in the testis. Primary spermatocytes, Sertoli cells, Leydig cells and intra-testicular vessels showed the expression of PARP pathway related proteins. In conclusion, *Trans*-Resveratrol mitigates T1DM-induced sperm abnormality and DNA damage, but does not significantly modulate PARP signaling pathway, except the SirT1 expression, in the rat testis.

1. Introduction

Diabetes-induced oxidative stress in men and in animals causes testicular dysfunction, disrupts hypothalamic-pituitary-gonadal axis, and induces erectile dysfunction and retrograde ejaculation (Jangir and Jain, 2014; Kilarkaje et al., 2014; Rocha et al., 2014; Vendramini et al., 2014). In addition, diabetes induces structural changes in testis such as apoptosis and degeneration of germ cells, epithelial sloughing, seminiferous tubular shrinkage and tubular atrophy. The oxidative stress induces DNA single- and double-strand breaks in the testis and in spermatozoa (Mallidis et al., 2007; Agbaje et al., 2008). Type 1 diabetes mellitus (T1DM) inhibits the expression of poly (ADP-ribose) polymerase 1 (PARP1) protein in rat testes in a seminiferous epithelial stage-dependent manner along with increases in oxidation of deoxyguanosine base, p53-p21$^{CIP1/WAF1}$ signaling and apoptosis of germ cells (Kilarkaje et al., 2014; Kilarkaje and Al-Bader, 2015). DNA fragmentation in spermatozoa of diabetic men correlates with an increase in the formation of advanced glycation end products and their receptors (Mallidis et al., 2007; Karimi et al., 2012). Thus, diabetes-induced disruptions in intracellular signaling pathways in testicular germ cells and in somatic cells may play key roles in the genesis of reproductive impairments in both animals and humans. As several molecular mechanisms underlying diabetes-induced testicular dysfunction are becoming clearer than ever before, more and more studies are undertaken to counteract the dysfunction using therapeutic drugs or antioxidants.

Resveratrol- an antioxidant and a non-flavonoid polyphenolic compound (3, 5, 4`-trihydroxystilbene)- is found mainly in red grape skin (Amri et al., 2012; Lee et al., 2014), but also in peanuts, berries, and legumes and can be extracted from several other natural sources (Kundu et al., 2006; Goswami and Das, 2009). Upon administration, Resveratrol is absorbed well and reaches to its peak plasma concentration approximately at 1.5 h post-dose, and gets metabolized in the liver to Resveratrol-glucuronides and Resveratrol-monosulfates, whose plasma concentrations are much more than that of the parent

* Corresponding author at: Department of Anatomy, Faculty of Medicine, Kuwait University, PO Box 24923, Safat 13110, Kuwait.
E-mail address: knarayana@hsc.edu.kw (N. Kilarkaje).

http://dx.doi.org/10.1016/j.taap.2016.09.023
0041-008X/

drug (Naderali, 2009), and the metabolites seem to be more important for the drug effects (Saiko et al., 2008). Resveratrol imparts antioxidant, anti-inflammatory, antiviral, chemo-preventive, neuroprotective and cardioprotective effects (Burkon and Somoza, 2008; Li et al., 2012). The antioxidant properties of Resveratrol are due to its ability to scavenge hydroxyl radicals, superoxide anion and hydrogen peroxide, and to up-regulate endogenous antioxidant production (Li et al., 2012). Resveratrol induces the production of superoxide dismutase, catalase and glutathione peroxidase enzymes in different cell types by modulating nuclear factor E2-related factor-2 (Nrf2) and silent information regulator two-1 (sirtuin 1 or SirT1) signaling pathways (Li et al., 2012). In addition, when given to animals, Resveratrol improves insulin sensitivity, glucose transporter type 4 translocation, and regulates enzymes involved in glucose metabolism, 5' adenosine monophosphate-activated protein kinase, decreases adipogenic genes, and prevents end organ damage (Bagul and Banerjee, 2015). Moreover, in both humans and animals, Resveratrol regulates numerous intracellular signaling pathways resulting in disparate cellular functional alterations with distinct clinical implications. Although Resveratrol is still not an approved drug for use in humans, it has shown beneficial effects in patients suffering from a wide variety of diseases including arthritis, cancer, diabetes, epilepsy, proliferative retinopathy and renal failure (Bagul and Banerjee, 2015; Lin et al., 2016; Liu et al., 2016; Lukawski et al., 2016).

Effects of Resveratrol on human male reproductive functions are not clearly known although it arguably improves the sperm quality in men (Garcez et al., 2010; Collodel et al., 2011). Resveratrol passes through the blood-testis barrier and imparts its protective effects in the testis (Aitken and Roman, 2008). Previous studies in animals have shown that Resveratrol successfully ameliorates either chemicals/drugs- or varieties of experiments-induced testicular structural changes, impaired sperm quality, cell death and disrupted reproductive functions (Ourique et al., 2013; Chirumbolo, 2015; Faid et al., 2015; Li et al., 2015; Banerjee et al., 2016). Resveratrol supplementation to normal rats resulted in enhanced activity of hypothalamic-pituitary-gonadal axis associated with improved sperm quality and quantity (Juan et al., 2005). However, it is not known whether or not Resveratrol has any modulatory effects on T1DM-induced sperm abnormalities, DNA damage and PARP signaling pathway in the testis, as the latter pathway is involved in DNA damage repair processes (Agarwal et al., 2009).

The PARPs are a group of nuclear enzymes, which gets activated in response to DNA damage (Agarwal et al., 2009). The PARP superfamily contains 18 isoforms and they use nicotinamide dinucleotide (NAD^+) as a substrate to synthesize poly (ADP-ribose) (pADPr) polymers, which attach to acceptor proteins, including PARP itself (Belenky et al., 2007; Rouleau et al., 2010; Zaja et al., 2012; Swindall et al., 2013; Vyas et al., 2013). Poly (ADP-ribosyl)ation of proteins, a type of post-translational modification, is stimulated by DNA strand breaks, especially that of nuclear proteins needed for the attraction of DNA damage repair enzymes to damage sites (Ame et al., 2004; Wang et al., 2009; Mashimo et al., 2013). The PARPs are central to a process of induction of a caspase-3-independent, apoptosis-inducing factor (AIF)-mediated type of cell death called parthanatos (Wang et al., 2009; Galluzzi et al., 2012), which, for example, has been observed in neurons after glutamate-induced excitotoxicity or in the heart under diabetic and ischemic conditions (Wang et al., 2009; Mashimo et al., 2013; Morales et al., 2014; Batnasan et al., 2015). High intracellular levels of PARPs, especially that of PARP1, not only increase pADPr polymer levels and deplete NAD^+ and ATP concentrations, but also stimulate the release of AIF to cytosol from mitochondrial inter-membranous spaces (Galluzzi et al., 2012; Isabelle et al., 2012; Lee et al., 2013; Mashimo et al., 2013). Therefore, the AIF is yet another protein that is central to the induction of parthanatos; in fact, its nuclear re-localization is essential for parthanatos (Susin et al., 1999; Ame et al., 2004; Galluzzi et al., 2012; Mashimo et al., 2013). Unlike apoptosis, parthanatos does not induce apoptotic body formation or small scale DNA fragmentation; and unlike necrosis, it does not induce cell swelling (Wang et al., 2009). However, although diabetes is known to induce apoptosis of testicular cells, it is not known whether or not parthanatos underlies testicular dysfunction in diabetic men and in diabetic animals.

In view of this, the present study was designed to investigate modulatory effects of *Trans*-Resveratrol on T1DM-induced 1) sperm abnormality, 2) DNA damage in testis and mature spermatozoa, and 3) PARP signaling pathway and parthanatos in the rat testis.

2. Materials and Methods

2.1. Animals

Male Wistar rats (13–15 week-old) were procured from the Animal Resources Center of Kuwait University. The rats were housed in plastic cages with sterilized sawdust bedding under standard laboratory conditions (temperature, 21–25 °C; 50% humidity; 12:12 light: dark cycle). They were fed with laboratory chow and tap water *ad libitum*. This experiment followed ARRIVE (Animal Research: Reporting of *in Vivo* Experiments) Guidelines and Kuwait University Guidelines for the care and use of animals in experiments and was approved by the Institutional Animal Ethics Committee.

2.2. Experimental design and sample collection

The rats were divided into four groups (n = 6–8/group) as follows: group 1, control non-T1DM rats; group 2, *Trans*-Resveratrol-treated (5 mg/kg/day) non-T1DM control rats; group 3, T1DM rats; and group 4, *Trans*-Resveratrol-treated T1DM rats. A single intraperitoneal injection of Streptozotocin (50 mg/kg in 0.1M citrate buffer, pH 4.5), a selective pancreatic β-cell toxicant, was given to rats to induce T1DM. Hyperglycemia was confirmed 48 h after the injection and, thereafter every week to confirm the sustained hyperglycemia. *Trans*-Resveratrol (*Hereafter referred to as Resveratrol in the text*; cat#501-36-0; Cayman Chemicals, USA; purity ≥98%) was diluted in 1 mL dimethyl sulfoxide and was administered (5 days/week, once daily, ip) to rats of groups 2 and 4 starting from day 22 to 42 after the confirmation of the hyperglycemia. The dose-level of Resveratrol was selected based on several previous studies, which obtained beneficial effects of the drug in *in vivo* experimental systems (Silan, 2008; Palsamy et al., 2010; Palsamy and Subramanian, 2011; Faid et al., 2015). At present, it is not known how much of absorbed Resveratrol reaches the testis, but it appears that at least a major part of the absorbed amount of the drug reaches the testis as the drug provided beneficial effects against induced testicular damage. On day 42, the rats were euthanized by CO_2 inhalation. From all rats, the reproductive organs were removed by laparotomy and the testes were de-capsulated and stored in -80 °C until used for all assays, except for the immunohistochemistry for which the testes were fixed in the Bouin's fixative for 24 h. In previous studies, diabetes-induced histopathological changes in the testis have clearly been described (Kilarkaje et al., 2014; Faid et al., 2015), and the protective effects of Resveratrol on the induced testicular damage have recently been reported (Faid et al., 2015). Therefore, in the present study, histopathological and hormonal studies were not included.

2.3. Sperm morphology, sperm motility and sperm count

The epididymides were removed, caudae epididymis were separated, cleaned and minced in phosphate buffered saline (PBS; 1w/2v; pH 7.4; 37°C) and incubated for 5 min at room temperature. The suspension was filtered through an 80 μm pore-size nylon mesh and the filtrate was collected. The sperm morphology test, sperm motility and sperm count were performed according to the standard procedures described previously (Kilarkaje et al., 2014).