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Ram Sperm DNA Fragmentation under Hyperglycemia Conditions is Inhibited by Polyamines

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Abstract

Objective: To evaluate the *in-vitro* effect of the polyamines spermine, spermidine and putrescine on the epididymal ram sperm DNA under hyperglycemia conditions.

Methods: DNA was isolated from ram epididymal sperm. Purity and integrity of DNA, and the effect of polyamines were analyzed by UV spectroscopy and agarose gel electrophoresis. Vials containing 200 µg DNA with or without 10 mM polyamine solutions were incubated at 35°C for 30 or 180 days in the presence of 5.5 or 30 mM glucose, simulating normal and hyperglycemia conditions.

Results: Seen by the increase in chromicity at 260 nm, epididymal sperm DNA was glycated from 30 days of incubation with 30 mM glucose. The absorbance was increased 65.44, 91.59 and 21.86%, for sperm DNA from head, body and tail, respectively. When polyamines were added, sperm DNA was condensed and chromicity decreased. The hypochromicity induced by polyamines was greater for the sperm DNA from the body of the epididymis. The effect of the polyamines from highest to lowest was: Spermine > spermidine > putrescine. Electrophoresis analysis revealed that the epididymal sperm DNA was fragmented after 180 days of incubation not only with high glucose concentration but also with physiological concentration. DNA fragmentation was partially inhibited by polyamines, especially by spermine and spermidine, to a lesser extent by putrescine.

Conclusion: These results provide evidence that high glucose concentration such as 30 mM glucose causes epididymal sperm DNA glycation and fragmentation. Both processes were partially inhibited by polyamines. The protective effect of polyamines is a function of their polycation properties as show for spermine and spermidine.

Keywords: Epididymal ram sperm DNA; Hyperglycemia; Glycation; DNA fragmentation; Polyamines; Spermine; Spermidine; Putrescine

Introduction

As a group of metabolic diseases [1], diabetes mellitus affects the eye, heart, kidney, lower extremities and other organs and systems. The reproductive system does not escape to these complications. Several reproductive abnormalities are associated with this pathology, both in women and men. Among the most important sexual alterations observed in men as a consequence of type 1 or type 2 diabetes, are damage of the germinal epithelium, damage of the seminiferous ducts, thinning of the basement membrane, hyperplasia of the interstitial tissue, calcification of the vas deferens and decrease of the synthesis of androgens and gonadotropins. Sexual dysfunction is a predominant factor in diabetes mellitus, which includes a failure or decrease in libido, alterations in intercourse and retrograde ejaculation [2].

On the other hand, investigations have revealed that the characteristics of sperm DNA determine the reproductive capacity of the male, and it has been shown that fertility in man with diabetes can be diminished when DNA is fragmented [3,4], an aspect with relevance in assisted reproduction [5]. Sperm chromatin is a well-organized structure, its organization starts when long strands of DNA are gradually packaged resulting in chromosomal organization [6]. This process involves the participation of transition proteins and subsequently the synthesis of protamines during spermiogenesis and epididymal transit [7,8]. Information has shown that polyamines spermine, spermidine and putrescine are essential regulators of cell growth and gene expression, and they have been implicated in both mitosis and meiosis. In male reproduction, polyamine expression correlates with stages of spermatogenesis [9]. These polycations increase sperm motility among the many actions they perform [10].

It is known that under hyperglycemia conditions amino groups of nucleic acids can react non-enzymatically with glucose to form unstable Schiff bases that can then undergo the Amadori rearrangement to form irreversible advanced glycation end products (AGE´s) [11]. AGE´s are capable of damaging macromolecules either directly by modifying the structure and function or by interaction with a cell receptor namely the receptor for advanced glycation end product (RAGE) [12].The presence and distribution of the RAGE has been elegantly demonstrated in the reproductive tract of diabetic men [13], its high concentration in spermatozoa correlates directly with sperm nuclear DNA fragmentation [14]. DNA damage can be potentiated by lipid peroxidation products since it has been reported that the total concentration of AGEs is higher in the seminal plasma of diabetic men and that there is a correlation with high lipid peroxidation in sperm and seminal plasma [15].

In spite of the effect of hyperglycemia on sperm DNA in diabetes is known and the role of polyamines in the reproductive tract widely documented [9,16,17], there are not reports related to the effect of polyamines and long term hyperglycemia on DNA integrity in the process of epididymal sperm maturation.

This study was designed to evaluate the *in-vitro* effect of polyamines on epidydimal sperm DNA using low and high concentrations of glucose simulating physiological and chronic hyperglycemia conditions. The results provide evidence that epididymal sperm DNA fragmentation is inhibited by polyamines especially by spermine and spermidine.

Materials and Methods

Chemicals

Polyamines Putrescine dihydrochloride, Spermidine trihydrochloride, Spermine tetrahydrochloride, D-(+)-Glucose, Ficoll-Paque, Trizma base, Sodium dodecyl sulfate (SDS), Dithiothreitol (DTT), Ethylenediaminetetraacetic acid (EDTA), Ethidium bromide, and Agarose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol was purchased from Merck-Mexico, S. A. All other chemicals were of analytical grade.

Spermatozoa collection and DNA extraction

Epididymal spermatozoa were obtained from mature rams killed at slaughterhouse as described previously [18]. The epididymus were dissected from the testis (n=4) and sectioned in caput, body and tail approximately 60 minutes after orchiectomy. During this time span, were kept in ice. Each fraction was minced and placed separately in 20 ml of cold phosphate buffer 0.01 M, pH 7.4 and gauze filtered. To avoid contamination by lymphocyte and epithelial cells, samples were filtered through a Ficoll-Paque gradient under sterility conditions. Cell suspension purity was confirmed using optical microscopy. Sperm cells were recovered after centrifugation at 3000 rpm for 10 minutes at room temperature, and washed twice with 4 ml phosphate buffer. The pellets were treated with 10 ml buffer Tris-EDTA-SDS-DTT (0.15 M-0.015 M-2%-1 mM, respectively) for DNA extraction [19]. This procedure involves the elimination of denatured proteins, lipids and RNA. Finally, DNA was precipitated with cold ethanol and dehydrated at 37°C. To experimental assays, DNA was dissolved in 0.154 M NaCl and quantified by ultraviolet spectroscopy in a scanning interval of 200-400 nm using a Lambda 25 Perkin Elmer spectrophotometer. Absorbance at 260 nm equal to 1.0 was equivalent to 50 µg DNA/ml. Integrity of DNA was analyzed by using 1% agarose gel electrophoresis with bromophenol blue as indicator (Mini-Sub Cell GT BIO-RAD) during 40 minutes, 80 V, and ethydium bromide as a fluorescent marker. An UV transiluminator was used to analyze DNA samples. The presence of DNA of high molecular weight and not of small fragments was visualized in the photo-documenter (Digital Imaging System IS-1000 Alpha Innotech Corporation), the images were captured in the program Alphamager 3.24I.

Experimental assays

In order to compare the glucose effect on sperm DNA isolated from different regions of epididymus, 5.5 mM and 30 mM glucose simulating physiological and hyperglycaemic conditions were tested. When the effect of polyamines was evaluated 10 mM spermine, spermidine or putrescine and 200 µg DNA in each case were used. Analysis of sperm DNA by UV spectroscopy (400 to 200 nm) was as follow: Vials 1-3, sperm DNA from head, body and tail in saline solution (Controls).

Vials 4-6, sperm DNA from head, body and tail + 5.5 mM glucose. Vials 7-9, sperm DNA from head, body and tail + 30 mM glucose. Vials 10-12, sperm DNA from head+spermine, spermidine and putrescine. Vials 13-15, sperm DNA from head +5.5 mM glucose+spermine, spermidine and putrescine. Vials 16-18, sperm DNA from head+30 mM glucose+spermine, spermidine and putrescine. Vials 19-21, sperm DNA from body +spermine, spermidine and putrescine. Vials 22-24, sperm DNA from body+5.5 mM glucose+spermine, spermidine and putrescine. Vials 25-27, sperm DNA from body+30 mM glucose +spermine, spermidine and putrescine. Vials 28-30, sperm DNA from tail+spermine, spermidine, and putrescine. Vials 31-33, sperm DNA from tail+5.5 mM glucose+spermine, spermidine and putrescine. Vials 34-36, sperm DNA from tail+30 mM glucose+spermine, spermidine and putrescine. Once the DNA spectrum was recorded in the different experimental conditions (Day 0), the vials were incubated in the dark at 35°C for 30 days (experimental). A similar series of vials were incubated under the same conditions for 180 days. Samples of each experimental condition were analized by electrophoresis.

Results

UV spectroscopy

Effect of 5.5 mM and 30 mM glucose: The absorbance recorded at 260 nm of the sperm DNA obtained from the different regions of the epididymis exposed to 5.5 mM glucose (vials 4-6) and 30 mM glucose (vials 7-9) are sumMarized in Table 1. While no change in baseline time was observed in sperm DNA samples, hyperchromicity after 30 days of incubation was observed in the sperm DNA of the body and tail

of the epididymis when 5.5 mM glucose was added to the incubation mixture. When incubation was carried out with 30 mM glucose, the absorbance increased considerably in the sperm DNA of the three regions of the epididymis. Seen by the hyperchromicity of the sperm DNA, the concentration of 30 mM glucose seemed to affect more the DNA obtained from head spermatozoa, later to the sperm DNA of the body and to a lesser extent to the sperm DNA of the tail of the epididymis.

The comparison of both glucose concentrations on sperm DNA from head of epididymus is shown in Figure 1. Apart of changes in absorbance at 260 nm, modification of glycosylation-associated chromophore development can be seen with absorbance near to 340 nm. Formation of this chromophore may be related to residual proteins DNA associated and products of glucose autoxidation as it has been demonstrated by using bovine serum albumin and glucose [20]. In our experience, drastic treatments that include the use of phenol do not completely eliminate proteins that remain tightly bound to DNA.

Table 1: Absorbance at 260 nm of epididymal sperm DNA at the Day 0 and after 30 days of incubation in the presence of 5.5 mM and 30 mM glucose.

*Sperm DNA source	5.5 mM Glucose		30 mM Glucose	
	Basal	Experimental	Basal	Experimental
Head	0.1845	0.1878	0.1907	0.3155
Body	0.1493	0.2323	0.1154	0.2211
Tail	0.2305	0.333	0.2346	0.2859

Hyperchromicity was 1.78, 55.59 and 44.46% for sperm DNA from head, body and tail, respectively, in the presence of 5.5 mM glucose. When sperm DNA was exposed to 30 mM glucose, the values were 65.44, 91.59 and 21.86% for sperm DNA from head, body and tail, respectively. (S. D. was omitted in Tables 1-4, but ranked within 1-4% of the mean). *200 µg DNA were used for each experimental condition (n=3) from Table 1- 4.

Table 2: Absorbance values after 30-day incubation of sperm DNA from different regions of the epididymis with 10 mM polyamines.

Polyamines						
*Sperm DNA source	Spermine		Spermidine		Putrescine	
	Basal	Experimental	Basal	Experimental	Basal	Experimental
Head	0.184	0.1909	0.2276	0.2676	0.1742	0.2531
Body	0.1347	0.1225	0.1627	0.1922	0.1045	0.1821
Tail	0.2396	0.1933	0.2554	0.2692	0.2336	0.2941

Hyperchromicity was 3.75, -9.06 (hypochromicity) and -19.33 (hypochromicity)% for sperm DNA from head, body and tail, respectively, in the presence of spermine. For spermidine, the values were 17.57, 18.13 and 5.40% for sperm DNA from head, body and tail, respectively. When putrescine was added, increase was 45.29, 74.25, 25.89% for sperm DNA from head, body and tail, respectively.

Table 3: Absorbance values after 30-day incubation of sperm DNA from different regions of the epididymis with 5.5 mM glucose and polyamines.

5.5 mM Glucose +10 mM Polyamines						
*Sperm DNA source	Spermine		Spermidine		Putrescine	
	Basal	Experimental	Basal	Experimental	Basal	Experimental
Head	0.1965	0.2314	0.2219	0.2526	0.1849	0.2141
Body	0.112	0.1482	0.1549	0.1878	0.0976	0.2035
Tail	0.2751	0.2538	0.2763	0.3094	0.2203	0.2467

Hyperchromicity was 17.14, 32.32, -7.75 (hypocromicity)% for sperm DNA from head, body and tail, respectively, in the presence of 5.5 mM glucose plus spermine. For spermidine increase was 13.83, 21.23, 11.97% for sperm DNA from head,

body and tail, respectively. For putrescine values were 15.79, 108.50, 11.98% for sperm DNA from head, body and tail, respectively.

Table 4: Absorbance recorded at 260 nm after 30-day incubation sperm DNA treated with 30 mM glucose and polyamines.

30 mM Glucose +10 mM Polyamines						
*Sperm DNA source	Spermine		Spermidine		Putrescine	
	Basal	Experimental	Basal	Experimental	Basal	Experimental
Head	0.1957	0.2482	0.246	0.1796	0.1877	0.2359
Body	0.1032	0.1286	0.1647	0.1941	0.1149	0.1865
Tail	0.244	0.2396	0.2526	0.279	0.255	0.252

Hyperchromicity was 26.82, 24.6, -1.81(hypochromicity)% for sperm DNA from head, body and tail, respectively, in the presence of 30 mM glucose plus 10 mM spermine. For spermidine, the chromicity was -27.00 (decrement), 17.85, 10.45% for sperm DNA from head, body and tail, respectively. For putrescine were 25.67, 62.31, 98.82% for sperm DNA from head, body and tail, respectively.

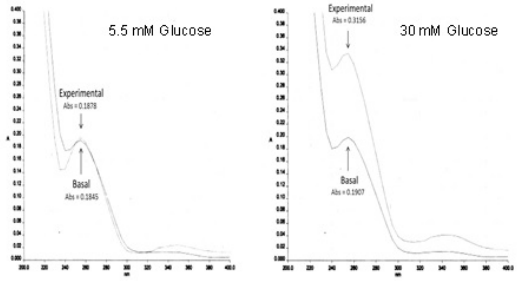

Figure 1: Effect of 5.5 mM (vial 4) and 30 mM glucose (vial 7) on the spectral properties of sperm DNA obtained from head of epididymus after 30 days of incubation at 35°C.

Effect of polyamines: When the sperm DNA obtained from the head of epididymis was incubated for 30 days with polyamines, increase in absorbance was observed mainly for DNA samples containing spermidine and putrescine (Table 2 and Figure 2), whereas in those samples containing spermine the increase in absorbance was almost nil or even lower as occurred for the sperm DNA obtained from the body (Figure 3). Sperm DNA obtained from the tail of epididymis was just lower for spermine under experimental conditions, compared with spermidine and putrescine (Table 2).

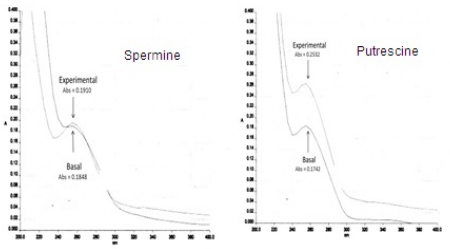

Figure 2: DNA of epididymal head sperm incubated for 30 days with 10 mM spermine (vial 10) and 10 mM putrescine (vial 12). The addition of spermidine caused an intermediate increase in the absorbance between spermine and putrescine (vial 11, spectral record not included).

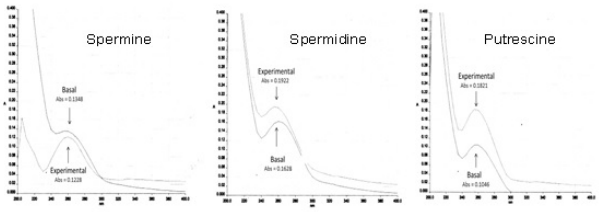

Figure 3: Sperm DNA from body of the epididymus incubated for 30 days with 10 mM polyamines (vials 19-21).

Effect of 5.5 mM glucose plus polyamines (30 days)

When sperm DNA obtained from different regions of the epididymis was incubated for 30 days with 5.5 mM glucose and 10 mM spermine, spermidine and putrescine, no significant changes were observed in absorbance recorded at 260 nm (Table 3), or in the spectral signals for the sperm DNA of the head of the epididymis (vials 13-15, data not shown).

However, when the sperm DNA obtained from the epididymis body was incubated under the same conditions, the absorbance tended to decrease, and the absorption spectra showed some changes particularly for spermine and spermidine (Figure 4).

DNA from spermatozoa obtained from the tail of the epididymis incubated under the same conditions, had apparently normal patterns similar to those shown by DNA in the presence of polyamines (Table 2).

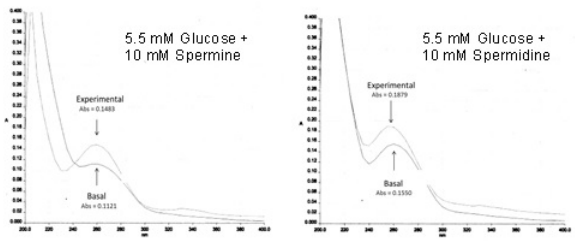

Figure 4: Epididymal body sperm DNA incubated 30 days with 5.5 mM glucose and 10 mM spermine and spermidine (vials 22 and 23).

Effect of 30 mM glucose plus polyamines (30 days)

Important changes were observed in absorbance recorded at 260 nm (Table 4) when sperm DNA obtained from different regions of the epididymis was treated for 30 days with 30 mM glucose and polyamines. Figure 5 shows spectral signals for the sperm DNA of the head of the epididymis when it was treated with 10 mM spermine, spermidine and putrescine (vials 16-18).

In Figure 6, the effect of spermine on the epididymal sperm DNA is compared, the main changes were observed for sperm DNA from the head and body of the epididymis, while the sperm DNA from the tail of the epididymis showed no major changes.

In Figure 7, the effect of 30 mM glucose plus 10 mM polyamines on epididymal body sperm DNA incubated for 30 days is shown (vials 25,26,27 respectively).

When the effect of 30 mM glucose plus polyamines was studied on epididymal tail sperm DNA, increase in absorbance at 260 nm was no observed for spermine (Figure 6, vial 34). Increase in absorbance was also avoided by spermidine and putrescine, but to a lesser extent (vials 35 and 36, data not shown).

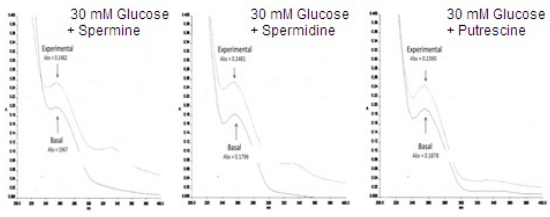

Figure 5: Sperm DNA from epididymal head incubated 30 days with 30 mM glucose and 10 mM polyamines (vials 16-18).

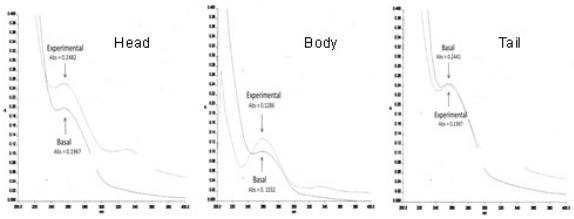

Figure 6: Comparison of 30-day incubation of epididymal sperm DNA with 30 mM glucose and 10 mM spermine (vials 16, 25, 34, respectively).

Effect of 5.5 and 30 mM glucose plus polyamines (180 days)

Sperm DNA samples treated with both glucose concentrations and polyamines incubated for 180 days were analyzed by UV spectroscopy in the same way to those treated for 30 days, with similar results (data not shown). However, when the different samples were analyzed by electrophoresis, important changes on the DNA integrity were observed (Figure 8).

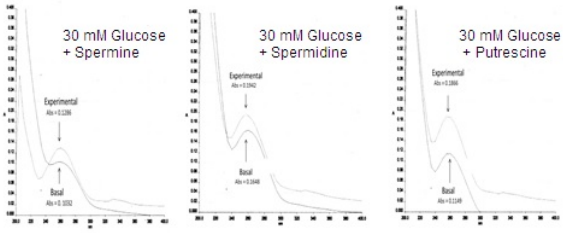

Figure 7: Comparison of the effect of 30 mM glucose plus 10 mM spermine, spermidine and putrescine on epididymal body sperm DNA incubated during 30 days (vials 25,26,27, respectively).

Electrophoresis analysis

Figure 8 shows the integrity of newly isolated sperm DNA obtained from epididymal tail. As can be seen, the DNA could be obtained highly polymerized using a simple and rapid methodology (Abs 260/Abs 280=1.8).

(A) Fragmentation was not observed in any of the cases when DNA was mixed with 5.5 mM or 30 mM glucose and imMediately subjected to electrophoresis (Day 0).

(B) No apparent change was also observed by electrophoresis when 10 mM spermine, spermidine or putrescine was added to DNA at Day 0. No change in the electrophoresis pattern of DNA could be observed at 30 days in the presence of 5.5 mM or 30 mM glucose or when the polyamines were added to the incubation mixture (data not shown).

However, DNA was fragmented when it was incubated for 180 days in the presence of 5.5 mM or 30 mM glucose. The degree of DNA fragmentation was dependent on the epididymal region from which the sperm cells were isolated. When polyamines were added to the incubation mixture, DNA fragmentation was partially inhibited.

(C). The inhibitory effect of polyamines was stressed for spermine, followed by spermidine and to a lesser extent to by putrescine.

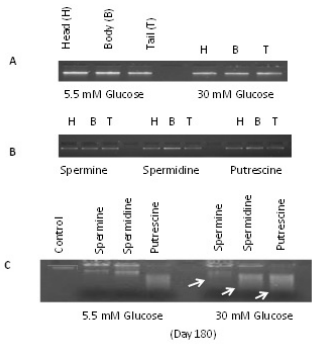

Figure 8: (A) Epididymal sperm DNA (head, body and tail) incubated for 30 days with 5.5 and 30 mM glucose. (B) Sperm DNA with 10 mM polyamines. (C) A combination of epididymal tail sperm DNA plus glucose and polyamines was incubated for 180 days. A DNA sample not treated with glucose or polyamines was included (Control).

Discussion

In this study, the short and long term effect of two glucose concentrations and polyamines on the integrity of epididymal sperm DNA was evaluated by two ways; UV spectroscopy and agarose gel electrophoresis. When the sperm DNA was exposed to both; the physiological and a high glucose concentration over a period of 30 days, changes in its spectroscopic properties at 260 nm were observed mainly with 30 mM glucose (Figure 1). When it was analyzed by agarose gel electrophoresis, sperm DNA fragmentation was not observed (Figure 8). However, when DNA was exposed for 180 days to both glucose concentrations not only high glucose concentration caused sperm DNA fragmentation but also physiological concentration (Figure 8). This may be explained since it is known that advanced glycation end products formation is a time-comsuming process and involves non-enzymatic reactions between carbohydrates and proteins, lipids or nucleic acids [11]. Under physiological conditions, levels of Schiff base and Amadori compound reach equilibrium after approximately 4 weeks. Then, through subsequent molecular rearrangements, which include reactions of dehydration, condensation, oxidation and cyclization; processes that take months, a heterogeneous group of fluorescent and dark brown are formed [21,22]. Most of the reactions at this stage are observed under oxidative conditions [23], and it is also known that high levels of phosphate concentrations increase the rates of reactions in the second stage [24].

The spectral changes observed with 30 mM glucose after incubation for 30 or 180 days are associated with DNA glycation that occurs by the autoxidation of glucose, as it was demonstrated for in vitro glycation of bovine serum albumin by glucose in the presence of transition metals, which occurs in relatively short periods of time such as 6 days [20]. Has been reported that although the primary amino groups of the nucleotides are chemically less reactive toward the reducing carbohydrates than the amino group of the lysine, glycation of the nitrogenous bases constituting the nucleic acids has been observed which causes abnormalities in the DNA pattern [25]. *In-vitro* studies with DNA from human blood and glucose concentrations in a range of 50 to 200 mM have shown that some parameters such as melting temperature and the concentration of Amadori products were increased after 30 days of incubation, in addition to changes in DNA chromicity [26].

In our study, the hyperchromicity shown in Figure 1 can be attributed to the AGEs formation, among them to N^2-(1-carboxymethyl-2'-deoxyguanosine (CMdG) and N^2-(1-carboxyethyl)-2'-deoxyguanosine (CEdG), adducts that are formed by glycation of DNA with glucose through the formation of glyoxal and methylglyoxal [27-30], and estimated to be more stable. It has been reported that the major nucleotide AGEs are imidazopurinone derivates [30,31]. The formation of these compounds is widely documented [32]. In addition to this, recently was reported that plasma 7,8-dihydro-8-oxo-2´-deoxyguanosine (8-OxodG) and 3-(2´-deoxyribosyl)-6,7-dihydro-6,7-dihydroxyimidazo-[2,3-b]purin-9(8)one (GdG) were increased 2-fold and 6-fold, respectively, in patients with type 2 diabetes, thus as urinary excretion rates of 8-OxodG, GdG, 3-(2´-deoxyribosyl)-6,7-dihydro-6,7-dihydroxy-6/7-methylimidazo-[2,3-b]purine-9(8)one (MGdG), and N^2-(1,R/S-Carboxyethyl)-deoxyguanosine and R/S epimers dG: deoxyguanosine (CEdG) which were increased 28-fold, 10-fold, 2-fold, and 2-fold, respectively, in patients with type 2 diabetes compared to healthy controls [33]. Nephropathy was associated with increased plasma 8-OxodG and increased urinary GdG and CEdG.

As shown in Figure 1, the hyperchromicity observed with 30 mM glucose occurred with the sperm DNA of the three regions of the epididymis. The hyperchromicity seemed to decrease as the sperm cell matures, since it was different in each case, being higher for the sperm DNA obtained from the head of the epididymis, followed by the DNA of the middle piece and smaller for the sperm DNA of the tail of the epididymis.

When the DNA was incubated with polyamines, the hyperchromicity decreased considerably (Figure 2). This can be due to the interaction between the phosphate groups of the DNA and the polyamines. Spermine, which has four amino groups, was the molecule that caused the greatest hypochromicity, followed by spermidine and putrescine, which have three and two amine groups, respectively. The effect of the polyamines was different when they were incubated with the sperm DNA of the head, body and tail of the epididymis. A condesation effect appeared to have exerted on the DNA particularly that obtained from the middle piece, which showed even lower chromicity than the sample under basal conditions (Figure 3). The order of hypochromicity induced by polyamines

from highest to lowest was: spermine>spermidine>putrescine. The addition of polyamines also decreased the hyperchromicity caused by 5.5 mM glucose on epididymal body sperm DNA (Figure 4) and 30 mM glucose on sperm DNA from head (Figure 5) incubated for 30 days.

Figure 6 compares the effect of spermine on sperm DNA from the three regions of the epididymis incubated for 30 days with 30 mM glucose. The spermine showed an evident protective effect on the DNA of the three sources, seen through the changes in the chromicity. This effect was greater for the sperm DNA of the body of the epididymis, which was confirmed when comparing the effect of the three polyamines on the sperm DNA from the body of the epididymis (Figure 7). Since the polyamines are synthesized by somatic cells of the testis and are related to spermatogenesis [9], the body of the epididymis represents the intermediate point in the process of organizing sperm chromatin and where the replacement of basic proteins by polyamines could occur to end in the tail of the epididymis. As shown in Figure 8 for the sperm DNA obtained from the tail of the epididymis, the condensation induced by polyamines, especially spermine and spermidine, partially prevented fragmentation of the DNA.

Previous studies using protein-free DNA from calf thymus or chicken erythrocyte, extracted polynucleosomes isolated from chicken erythrocyte chromatin, and reassembled nucleosomes exposed to an oxygen radical generating system, showed that oxygen radicals extensively and randomly fragmented protein-free DNA, with double strand [34]. In contrast, polynucleosomes were converted to nucleosome zise-fragments by iron-ADP. Cleavage occurred only in bare areas where DNA is unassociated with histone. It has been stated that histones may provide a protective role for nuclear DNA in vivo. Certain areas of the genome, those not associated with histone protein, may be relatively unprotected against mutagenic damage, and actively transcribing genes, with more “open” chromatin strucucture and decrease histone binding to DNA may become preferred targets for oxidant damage [35].

Other *in-vitro* studies have shown a close association of polyamines with DNA [36], neutralizing at least in part its negative charges and stabilizing it [37,38]. Spermine and spermidine at physiological concentration are physical quenchers of singlet molecular oxygen [39]. They retard free-radical autoxidation processes [40]. DNA and calf thymus chromatin is condensed by spermine, spermidine and some triamines analogs of polyamines and diamines in a similar way [41]. The stoichiometry of polyamine binding at which condensation of chromatin is completed is found to be two polyamine molecules per DNA turn. The extent of neutralization of the DNA phosphates by histones in these compact fibers is estimated to be about 55% [41].

From studies with human genomic DNA isolated from peripheral blood leukocytes it has been shown that nuclear aggregates of polyamines are able to preserve the genomic DNA from DNAase 1-dependent degradation, and it has been stated that these aggregates of polyamines are the sole biologically active forms by which polyamines physiologically interact with and protect genomic DNA [42].

Recently was found that polyamines self-assemble with phosphate ions in the cell nucleus and generate three forms of compounds referred to as nuclear aggregates of polyamines, which interact with genomic DNA. These nuclear aggregates have a cyclic structure, and in the presence of DNA, they form a tube-like arrangement around the double helix suggesting a decisive role of natural nuclear aggregates of polyamines in regulating important aspects of DNA physiology, such as conformation, protection and packaging, thus suggesting a new vision of the functions that polyamines accomplish in the cell nucleus [43]. Today is recognized the site- and structure-specific binding of polyamines to DNA apart of their binding by electrostatic interaction, which results in nanoparticles of approximatately 100 nm diameter [43,44].

Conclusions

In conclusion, these results show that epididymal sperm DNA was affected by 30 mM glucose causing its glycation and fragmentation in a very short time. Both processes were partially inhibited by polyamines.

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