Effects of Ni(II) and Cu(II) on DNA interaction with the N-terminal sequence of human protamine P2: enhancement of binding and mediation of oxidative DNA strand scission and base damage

Rongti Liang, Sema Senturker1, Xianglin Shi2, Wojciech Bal3, Miral Dizdaroglu1 and Kazimierz S.Kasprzak4

Laboratory of Comparative Carcinogenesis, National Cancer Institute, FCRDC, Frederick, MD 21702, USA. 1Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA, 2National Institute of Occupational Safety and Health, Morgantown, WV 26505, USA and 3Faculty of Chemistry, University of Wroclaw, 50-383, Wroclaw, Poland

Introduction

It has been suggested, based on epidemiology data, that paternal exposure to metal dusts and fumes, including welding fumes, may increase the incidence of cancer in the progeny (1,2). Nickel, a toxic and carcinogenic metal, is one of the primary suspects (3), and sperm DNA may be considered as its most likely target. Indeed, experimental exposure of male mice to Ni(II) chloride resulted in a transient accumulation of Ni(II) in testes accompanied by reduced sperm count and chromosomal aberrations (4,5). Also, Ni(II) treatment slightly increased Cu(II) levels in the testes (4). Possible mechanisms of those aberrations would involve DNA damage by reactive oxygen species (e.g. base alteration, crosslinking, strand cleavage and/or depurination) generated in Ni(II)- and Cu(II)-mediated redox reactions with participation of endogenous oxidants (6).

Sperm DNA is tightly packed with protamines that may sequester toxic transition metals and modulate oxidative damage. As found previously, human protamine P2 (HP2) has, indeed, a strong Ni(II) - and Cu(II)-binding amino acid motif at its N-terminus, Arg-Thr-His-Gly-, that should serve as a metal trap (7). Similar properties should also be expected for the homologous mouse P2, having the Arg-Gly-His- motif (8). We have also observed that Ni(II) and Cu(II) bound to a pentadecapeptide modeling this motif [Arg-Thr-His-Gly-Ser-His-Tyr-Arg-Arg-Arg-His-Cys-Ser-Arg-amide; HP21–15] were able to mediate oxidative DNA double-strand scission and generation of 8-oxo-2′-deoxyguanosine (8-oxo-dG) from free 2′-deoxyguanosine (dG) and from DNA by H2O2 (9). The aim of the present study was to further test the mechanistic involvement of HP21–15 in oxidative and non-oxidative attack of Ni(II) and Cu(II) on DNA. The oxidative effects investigated included the mediation of single- and double-strand scission and oxidation of all base residues in pUC19 plasmid DNA. Reactive oxygen species causing these effects were identified. The direct (non-oxidative) effects of Ni(II) and Cu(II) on the binding of HP21–15 to pUC19 were also studied.

Materials and methods

Materials

The HP21–15 peptide was custom synthesized by QBC (Hopkinton, MA). The purity of the peptide was verified by HPLC, mass spectrometry and above, the electron spin resonance/spin trap measurements revealed greater and more persistent generation of OH- and O2- like oxidants from H2O2 by the Ni(II)-HP21–15 complex than by the Cu(II)-HP21–15 complex (no O2- was detected). Both complexes were also found to bind to DNA more strongly than HP21–15 alone. The results indicate that protamine P2 is capable of binding Ni(II) and Cu(II) and, in this way, attenuating the mediation of oxidative DNA damage by Cu(II), but not Ni(II). The effects found may be mechanistically involved in the reproductive toxicity and carcinogenicity of metals.
potentiometry, and found to be >99%. Plasmid pUC19 DNA, calf thymus genomic DNA and restriction enzyme PsI I were purchased from New England Biolabs (Beverly, MA) and USB Specialty Biochemicals (Cleveland, OH), respectively. Nickel chloride (99.999% NiCl₂·6H₂O) and copper chloride (99.999% CuCl₂) were purchased from Aldrich (Milwaukee, WI). Sodium and potassium phosphates and other inorganic chemicals were purchased from J. T. Baker (Phillipsburg, NJ).

SeaKem LE Agarose was purchased from FMC BioProducts (Rockland, ME). Ethidium bromide was purchased from Bio-Rad ( Hercules, CA). Agarose gel loading dye solution (ultra pure grade) was purchased from Amresco (Solon, OH). DNA molecular weight standards and TBE buffer (0.1 M Tris, 0.09 M Tricine acid, 0.001 M EDTA, pH 8.4) were from Gibco BRL (Gaithersburg, MD). Formic acid (88%) was obtained from Malinkrodt (Paris, KY). Acetonitrile and bis(trimethylsilyl)-trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane were obtained from Pierce (Rockford, IL). The reference damaged DNA bases for instrument calibration, some of them labeled with stable isotopes 13C, 15N, and 35S, were acquired from Sigma (St Louis, MO). Merck/Isotopes (Montreal, Canada) or were synthesized by Dr V. Nelson from SAIC Frederick (Frederick, MD). 5,5-Dimethyl-1-pyrroline N-oxide (DMPO) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma. DMPO solutions were purified using activated charcoal until free radical impurities were removed as verified by electron spin resonance (ESR) spectroscopy.

Stock solutions were made with double distilled water and purified with Chelex-100 to reduce traces of transition metals prior to use (except for those of CuCl₂, NiCl₂, and H₂O₂). The buffer in which plasmid pUC19 DNA was supplied (10 mM Tris–HCl, 1 mM EDTA, pH 8.0), was changed into 50 mM phosphate-buffered 0.15 M saline (PBS), pH 7.4, using Centricron-30 centrifugal concentrators (Amicon, Beverly, MA).

DNA strand breakage

The covalently closed circular (ccc) pUC19 DNA of 1.5 mM phosphate group concentration was mixed in PBS ( pH 7.4) with various combinations of 8 µM HP2₁⁻₁₅, 8 µM NiCl₂, 8 µM CuCl₂ and 1 mM H₂O₂ (except for the metal concentration effect experiments in which 2–64 µM concentrations of metals were tested) and incubated for 16 h at 37°C in the dark. The reagents were mixed always in the same sequence, with DNA being added to PBS first, followed by HP2₁⁻₁₅, metal and H₂O₂. The experiments were carried out, at the very least, in duplicates. For strand breakage examination, the samples after treatment with premixed with agarose gel loading dye solution and subjected to electrophoresis (4 V/cm; 2 h) and a 1% agarose gel containing ethidium bromide in TBE buffer. Gels were destained and photographed were taken and processed with an IS-1000 Digital Imaging System (Alpha Innotech Corp.).

DNA base damage

Exposure of pUC19 to HP2₁⁻₁₅, Cu(II), Ni(II) and H₂O₂, as above, was followed by analysis of the damaged bases, using the gas chromatography/iso tope dilution–mass spectrometry with selected ion monitoring technique as described elsewhere (10,11). Briefly, the samples (in quadruplicates), containing 50 µg of pUC19 DNA each (before the incubation), were supplied with stable isotope-labeled internal standards, lyophilized and hydrolyzed with 0.5 ml of 60% formic acid in evacuated and sealed glass tubes for 30 min at 140°C. The hydrolysates were lyophilized and then trimethylsilylated under nitrogen in polytetrafluoroethylene-capped hypovials (Pierce) with 100 µl of a mixture of nitrogen-bubbled BSTFA and acetonitrile (4:1 v/v) by heating for 30 min at 120°C. Aliquots of such derivatized samples were then injected into a gas chromatograph column using an autosampler. A split ratio of 1:15 was used at the medium point. This resulted in ~0.25 µg of hydrolyzed and derivatized DNA going through the column during each analysis and a detection limit of ~3 pmol of base product/mg DNA. The quantification of DNA base products was performed by isotope dilution mass spectrometry using their stable isotope-labeled analogues as internal standards (10,11). The DNA amounts in the samples were also determined by this technique in addition to the use of absorbance measurement by UV spectroscopy. For this purpose, aliquots of dG⁻¹⁵N were added to DNA samples prior to hydrolysis. Upon hydrolysis, this compound yields guanine-¹⁵N₂, which is used for internal standard for guanine in DNA (12). The results of DNA determination by this method and by UV measurements correlated well with each other.

Oxygen radicals

The generation of radical species from H₂O₂ in our system was studied using the ESR spin trapping technique (13,14). Solutions containing 60 mM DMPO, 3 mM phosphate groups) calf thymus DNA in PBS, pH 7.4, plus various combinations of 2.5 mM H₂O₂, 0.2 mM HP2₁⁻₁₅ and 0.2 mM Cu(II) or Ni(II) in total volumes of 0.5 ml, were prepared at room temperature and placed in the ESR instrument 5 min or 24 h later. The measurements were made in a

Varian E9 ESR spectrometer and a flat cell assembly. The ESR spectrometer settings were: receiver gain, 2.5×10³; modulation amplitude, 0.5 G; magnetic field, 3500 G; 100 G; scan time, 4 min. Hyperfine splitting was measured to 0.1 G directly from magnetic field separations, using potassium tetraperoxo- chromate (K₂CrO₄; a gift from Dr K. Singh of West Virginia University) and DPPH as standards. An EPR DAP 2.0 program was used for data acquisition and analysis.

Metal effect on DNA-H₂O₂ binding

Solutions of HP2₁⁻₁₅ and Ni(II) or Cu(II) were mixed together in PBS, pH 7.4, and then combined with calf thymus DNA in PBS ( final concentrations: 120 µM DNA; 8 µM HP2₁⁻₁₅; 0, 2, 4 or 8 µM NiCl₂ or CuCl₂). They were incubated at 37°C for 16 h. The incubation mixtures were subjected to electrophoresis through a 0.3% agarose gel in TBE buffer after mixing with agarose gel loading dye solution and stained with ethidium bromide. Gel photographs were taken and processed with an IS-1000 Digital Imaging System (Alpha Innotech Corp.). Fractions of free and HP2₁⁻₁₅-bound DNA were quantified using a scanning function of the digital imaging system.

Results

DNA strand breakage

Treatment of ccc pUC19 DNA at pH 7.4 with H₂O₂ and various combinations of HP2₁⁻₁₅, Ni(II) and Cu(II) resulted in both single- and double-strand breakage, the extent of which strongly depended on the metal and the presence or absence of HP2₁⁻₁₅. These effects, examined with agarose gel electrophoresis, are shown in Figure 1. As can be judged from this figure, pUC19 used in this experiment contained predominantly the ccc form, but also some of the relaxed (nicked) and linearized forms of the plasmid, most likely a partially relaxed circular DNA. Amidase and horseradish peroxidase enzymes were used as references of double-strand cuts.

Fig. 1. Strand scission in ccc pUC19 plasmid exposed to H₂O₂ in the presence of equimolar concentrations of Ni(II), Cu(II) and HP2₁⁻₁₅ (8 µM) at combinations explained on the top of this figure. M, molecular weight markers; Rlx, Rlx, Lin and CCC, relaxed, linearized and circular forms of pUC19, respectively.

For strand breakage examination, the samples after treatment with premixed with agarose gel loading dye solution and subjected to electrophoresis (4 V/cm; 2 h) and a 1% agarose gel containing ethidium bromide in TBE buffer. Gels were destained and photographed were taken and processed with an IS-1000 Digital Imaging System (Alpha Innotech Corp.). Fractions of free and HP2₁⁻₁₅-bound DNA were quantified using a scanning function of the digital imaging system.

Results

DNA strand breakage

Treatment of ccc pUC19 DNA at pH 7.4 with H₂O₂ and various combinations of HP2₁⁻₁₅, Ni(II) and Cu(II) resulted in both single- and double-strand breakage, the extent of which strongly depended on the metal and the presence or absence of HP2₁⁻₁₅. These effects, examined with agarose gel electrophoresis, are shown in Figure 1. As can be judged from this figure, pUC19 used in this experiment contained predominantly the ccc form, but also some of the relaxed (nicked) and linearized forms of the plasmid, most likely a partially relaxed circular DNA.
resulting in a complete degradation of the plasmid to small pieces running off the gel (lane 7). The addition of HP2\textsubscript{1–15} tended to increase the enhancing effect of Ni(II) on H\textsubscript{2}O\textsubscript{2}-caused strand scission (note the vanishing ccc band in lane 11 versus lane 6), whereas the strand breaking effect of Cu(II)/H\textsubscript{2}O\textsubscript{2} was almost completely prevented (compare lanes 12 and 7). Figure 2 illustrates the effect of metals at various molar proportions to 8 µM HP2\textsubscript{1–15} (with other conditions unchanged) it is clear from this figure that HP2\textsubscript{1–15} sustained Ni(II)-mediated DNA nicking and double-strand breaking in a relatively wide range of Ni(II):HP2\textsubscript{1–15} molar ratios, varying from 0.25 to 8.0 (lanes 4–9 versus lane 1). In contrast, HP2\textsubscript{1–15} offered an apparently full protection against Cu(II)-mediated strand breaks up to the molar ratio of 1 (Figure 2, lanes 10–12), but was ineffective above that ratio (lanes 13 and 14).

**DNA base damage**

Exposure of ccc pUC19, as above, in addition to causing strand scission also resulted in oxidative DNA base damage. The results are presented in Figures 3 and 4. Both purine and pyrimidine bases were damaged, the overall treatment-related effects being very similar to those observed for the strand scission. Thus, the metals added to H\textsubscript{2}O\textsubscript{2} alone increased its oxidative activity towards all DNA bases, with Cu(II) being much more effective than Ni(II), except for the generation of FapyGua (lanes 3 and 4 in Figures 3 and 4). Also, judging by the percentage increases of the base products generation, caused by adding either metal to H\textsubscript{2}O\textsubscript{2}, the pyrimidines appeared to be more susceptible to Ni(II)- and Cu(II)-mediated oxidation than purines. HP2\textsubscript{1–15} had different effect on each metal. For Cu(II), it was a strong inhibition of the generation of all base products, though not to the background levels [except that of 2-hydroxyadenine (2-OH-Ade)] (compare lanes 6 and 2 in Figures 3 and 4). HP2\textsubscript{1–15} effects on Ni(II) were generally milder and mixed; they varied from a slight enhancement of 5-hydroxyhydratoin (5-OH-Hyd) and thymine glycol (ThyGlycol) to a various degree inhibition of yield of the other damaged bases (Figures 3 and 4, lane 3 versus lane 5). The generation of pyrimidine base products in the presence of HP2\textsubscript{1–15} was higher with Ni(II) than with Cu(II) (Figure 3).

**Oxygen radicals**

The ESR spectra, originating from solutions of DNA plus various combinations of H\textsubscript{2}O\textsubscript{2}, HP2\textsubscript{1–15}, Cu(II) or Ni(II) and a spin trap DMPO are shown in Figure 5. Solutions containing the reagents without H\textsubscript{2}O\textsubscript{2} were spectrally inactive (e.g. spectrum e in Figure 5). H\textsubscript{2}O\textsubscript{2} alone and in the presence of HP2\textsubscript{1–15} produced a very weak signal pattern resembling that of the hydroxyl radical OH·/DMPO adduct (spectra a and d). Strong spectra, typical for OH·/DMPO, were observed in mixtures containing DNA plus H\textsubscript{2}O\textsubscript{2} and Cu(II) or Ni(II) in the absence of HP2\textsubscript{1–15} produced a very weak signal pattern resembling that of the hydroxyl radical OH·/DMPO adduct (spectra a and d). Strong spectra, typical for OH·/DMPO, were observed in mixtures containing DNA plus H\textsubscript{2}O\textsubscript{2} and Cu(II) or Ni(II) in the absence of HP2\textsubscript{1–15} (spectra b and c in Figure 5); they were significantly weakened by the addition of HP2\textsubscript{1–15}, especially that of Cu(II) in comparison with Ni(II) (spectra f and h). Interestingly, the latter difference increased in samples incubated for 24 h, and in addition, a second signal typical for the O\textsuperscript{2–}/DMPO adduct appeared in the Ni(II)-containing mixture besides the OH·/DMPO signal (spectra g and i).

**Metal effect on DNA-HP2 binding**

As shown in Figure 6, both metals increased the binding of HP2\textsubscript{1–15} to calf thymus DNA, with Ni(II) being apparently, but not significantly, more effective than Cu(II).

**Discussion**

In our previous investigations we found that HP2\textsubscript{1–15}, modeling the N-terminus of human protamine P2, could strongly bind...
Ni(II) and Cu(II) (7) and sustain redox activity of both metals, though to a different extent, towards H$_2$O$_2$. This activity resulted in degradation of the peptide and oxidative damage to other molecules, including calf thymus DNA (9). The aim of the present study was to test further mechanistic details of the damage. In particular, we expected to find out (i) whether the HP2$_{1-15}$-metal complexes could mediate the production of both single- and double-strand breaks in DNA; (ii) if the strand breaks were accompanied by a wide spectrum of oxidative base damage; (iii) what kind of radical species, if any, were generated by HP2$_{1-15}$-metal complexes from H$_2$O$_2$ under conditions resulting in oxidative DNA damage; and (iv) whether there was any significant difference between Ni(II) and Cu(II) effect on DNA-HP2$_{1-15}$ binding.

Under the present experimental conditions, neither H$_2$O$_2$ nor the peptide and metals alone (in the presence of ambient oxygen) caused significant strand breaking and base damage in ccc DNA. As found in some additional experiments on ccc pUC19 pretreated with PstI (not shown), the same reagents were similarly inert towards the linear form of the plasmid. Hydrogen peroxide in the presence of HP2$_{1-15}$ slightly increased DNA strand breaking and base oxidation, especially the generation of 5-hydroxycytosine (5-OH-Cyt). This might be due to the attack of S-centered radicals from the cysteine residues of HP2$_{1-15}$ being oxidized by H$_2$O$_2$. Lack of evidence for such radical species in the corresponding ESR spectrum may result from their limited and site-specific formation at the peptide molecule tightly bound to DNA and thus inaccessible to the DMPO spin trap. Treatment of ccc pUC19 with H$_2$O$_2$...
in the presence of Ni(II) or Cu(II) resulted in a significantly greater oxidative damage, with Cu(II) being much more effective in this respect than Ni(II). The latter at 8 μM concentration produced predominantly single-strand breaks, whereas double-strand scission that would result in linearization (opening) of the plasmid and appearance of smaller linear DNA fragments was not evident. This remained in concordance with the lack of calf thymus degradation by H₂O₂ plus Ni(II) observed before (9). In contrast, under the present experimental conditions, the same molar concentration of Cu(II) resulted in a complete destruction of pUC19 DNA.

The above mentioned vast difference in the effectiveness of Ni(II) and Cu(II) to mediate DNA cleavage by H₂O₂ was also reflected in the catalysis of DNA base oxidation, but not in the magnitude of free radical generation by the respective metals, as measured by the spin trap technique. Consistent with its ‘Fenton metal’ character, Cu(II) appeared to mediate the base oxidation with greater efficiency than Ni(II), a non-Fenton metal. The activity of Ni(II) was surprising, since it may be rendered redox active under physiological conditions only after chelation by certain organic ligands, but not DNA (reviewed in refs 6 and 15). A great difference in activity between Ni(II) and Cu(II) might also have been expected in the generation of OH· (or OH·-mimicking metallo-oxo or -peroxo species). It was, however, not the case. As revealed in our ESR measurements, under the same conditions both metals generated comparable amounts of OH·. This discord between the magnitudes of DNA damage and free radical generation is difficult to comprehend. The most likely explanation is that Cu(II), being strongly coordinated by DNA bases, was likely to bring the radical generation closer to the bases and thus make their oxidation (but not the DMPO spin trapping) more efficient than in the case of Ni(II), which is more loosely bound to DNA phosphates (16,17). It is also possible that the unexpected pro-oxidative effects of the Ni(II)/H₂O₂ mixture versus DNA and the associated radical generation observed by ESR were entirely due to the presence of Ni(II)-chelating contaminants in the commercial DNA samples used in the present experiment (e.g. proteins and/or polyamines).

The addition of HP₂₁⁻¹₅ to the reaction had a dramatic effect on DNA oxidation by Cu(II) plus H₂O₂ and a relatively limited effect on the oxidation by Ni(II) plus H₂O₂. DNA strand breakage by the Cu(II)/H₂O₂ mixture was prevented by HP₂₁⁻¹₅ nearly completely, while base oxidation fell well below that caused by Cu(II)/H₂O₂ alone. In contrast, HP₂₁⁻¹₅ appeared to enhance both the single- and double-strand breakage as well as the generation of 5-OH-Hyd and ThyGlycol by the Ni(II)/H₂O₂ mixture. Its effect on levels of the other base products was more or less suppressive. As known from our previous studies, both metals are very strongly bound by HP₂₁⁻¹₅ (7). Hence, we may assume that DNA damage in the presence of HP₂₁⁻¹₅ depended solely on the interactions of DNA with, and redox properties of, the respective HP₂₁⁻¹₅-metal complexes. The present results indicate that chelation of Ni(II) by HP₂₁⁻¹₅ augmented oxidation catalysis by this metal toward DNA, while chelation of Cu(II) attenuated it. This conclusion stems from the presence (or absence) of both types of DNA damage studied and the appearance of the ESR spectra as well. Such vastly different effects of chelation on redox activity of transition metals have been reported before (18,19). Very interestingly, the increase of the ESR response to Ni(II) plus HP₂₁⁻¹₅ in time seemed to reflect the generally slow chelation kinetics and the enhancement by chelation of redox activity of this metal around pH 7, as observed previously in isolated chromatin (20). Alternatively, the progressing oxidative DNA damage might have resulted in gradual rearrangement of the substrates and products in a way enabling DMPO to scavenge free radicals more and more efficiently. The observed spin trap ESR response to Cu(II)-mediated oxidation indicates a strong suppression by HP₂₁⁻¹₅ of the generation of ‘free’ OH·-radical able to reach other molecules, such as DMPO and DNA. It is not clear whether Cu(II) chelated by HP₂₁⁻¹₅ retains some capacity to generate diffusible oxidants, as observed before for much higher concentrations of Cu(II) and HP₂₁⁻¹₅ (9), or the limited DNA nicking and oxidative base damage observed in this study in the presence of HP₂₁⁻¹₅-Cu(II) is due to Cu(II) exchanged between the HP₂₁⁻¹₅ and DNA base ligands. The results of experiment with increasing proportions of the added metal to a constant concentration of HP₂₁⁻¹₅ seem to support the notion that HP₂₁⁻¹₅ might be able to abrogate redox activity of Cu(II) completely, at least at micromolar and substoichiometric concentrations of the metal versus HP₂₁⁻¹₅. On the other hand, however, our previous results showing extensive damage of the HP₂₁⁻¹₅ ligand by Cu(II) plus H₂O₂ (9) indicate that HP₂₁⁻¹₅-bound Cu(II) retains redox activity. Hence, in the Cu(II)-HP₂₁⁻¹₅ complex, the metal-associated oxidant would preferentially attack the peptide, not DNA, while in the Ni(II)-HP₂₁⁻¹₅ complex, the peptide and DNA could both be targeted. These differences might be due to different ordering of the HP₂₁⁻¹₅ conformation by each metal (7) and, thus, possibly different spatial interaction of the complexes with DNA. The exact reactive species and mechanisms involved in the observed oxidation effects remain to be unveiled.

The binding of HP₂₁⁻¹₅ to DNA resulted in the formation of aggregates with lower electrophoretic mobility than that of free DNA. Both metals enhanced the formation of such aggregates. Ni(II) was apparently more active in this respect than Cu(II). However, this difference between the metals does not seem to be high enough to be solely responsible for the differences in the concurrent oxidative effects, discussed above. The dissimilar course of the metal concentration dependence of the binding may indicate different effects of Ni(II) and Cu(II) on HP₂₁⁻¹₅ conformation as concluded previously from the respective CD spectra (7).

The present study provides evidence that DNA cleavage with oxidants mediated by metals chelated by specific DNA-binding peptides, proposed for use as oxidative nuclease (19,21,22), may cause base oxidation as well. Thus, the products of such cleavage (DNA fragments, oligonucleotides) may not be perfect substitutes for enzymatic restriction fragments. Our own tests (unpublished) revealed lack of any pUC19 sequence specificity of the HP₂₁⁻¹₅-Cu(II) or HP₂₁⁻¹₅-Ni(II) complex-directed DNA cleavage.

Biological relevance of our results is associated with epidemiological evidence that certain types of childhood cancer are related to preconceptional paternal exposure to metals (1,2,23) and that some transition metals, including Cu(II), Ni(II) and Cr(III) are toxic to spermatozoa (4,5,24). Prostate P2, modeled here by HP₂₁⁻¹₅, is likely to be a major metal-binding protein in the sperm. As shown in this study, such binding appears to be capable, by itself, of altering the proteasome–DNA interaction and may thus affect proper assembly of DNA and P2 in the sperm and result in pathogenic effects. In addition, spermadites are rich in oxygen-metabolizing mitochondria from metal-protopine complexes and oxidative DNA damage
which H2O2 can leak out, react with a protamine-bound transition metal and generate reactive oxygen intermediates, including OH· radical and/or metal-associated oxidants (6,15). These species will, in turn, oxidize both the protamine and protamine-associated DNA and thus produce promutagenic damage that may result in sperm death or lead to birth defects and cancer in the progeny.

Acknowledgements

The authors wish to thank Dr Anthony J Fitzhugh for helpful critical comments on this manuscript and Ms Kathy Breeze for editorial assistance. This work was supported in part by the Polish Committee for Scientific Research (KBN) grant no. 6 PO4A 024 13 to W.B. Certain commercial equipment or materials identified in this paper in order to specify adequately the experimental procedure. Such specification does not imply recommendation or endorsement by the National Institute of Standards and Technology or National Institutes of Health, nor does it imply that the materials and equipment identified are necessarily the best available for the purpose.

References


Received October 28, 1998; revised December 29, 1998; accepted January 25, 1999