SUPPLEMENTAL INFORMATION

Electrophilic aldehydes generated by sperm metabolism activate mitochondrial reactive oxygen species generation and impair sperm function

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Supplementary Methods

Experimental determination of electrophilicity

A phosphate buffer solution containing disodium hydrogen phosphate (65 mM) and potassium dihydrogen phosphate (15 mM) was prepared. A 50 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) solution was prepared in the buffer solution and 1 M sodium hydroxide was added to correct the pH to 7.4. The GSH solution (25 mM) was freshly prepared for every experiment in the buffer solution. A stock solution of each compound was prepared in DMSO and further diluted in the buffer solution to a final range of 10 uM to 25 mM. The compound was then added to the GSH solution in the wells of a 96 well plate at T = 0 and after the designated time, DTNB was added and the absorbance was read at 376 nm. For NEM, the direct measurement of the electrophile via UV/vis spectroscopy was used instead of the DTNB reporter. NEM (150 µM) was added to the buffer solution (as above) and at T=0 GSH (150 µM) was added. The disappearance of NEM was followed by measuring the absorbance at 300 nm. The absorbance values were then used to calculate the half life and the second order reaction rate for each compound as per Bohme et al. (2009) by using a curve fitting approach based on a least sum of squares.

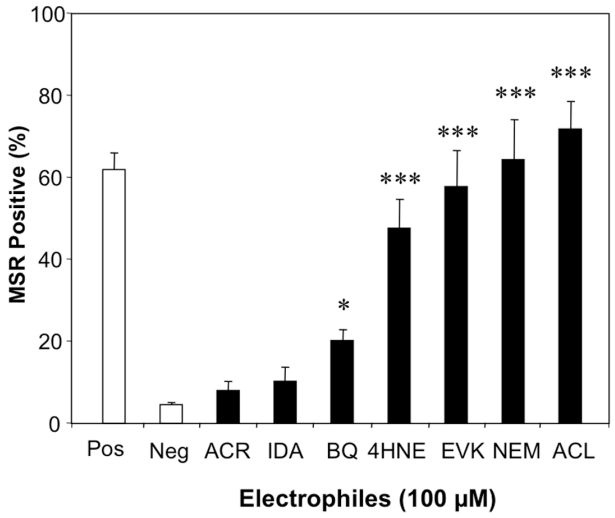
Measurement of succinic acid dehydrogenases and cytochrome oxidase activities

Succinate dehydrogenase activity was determined by measuring the reduction of 2,6-dichloroindophenol (DCIP) at 600 nm. Briefly, spermatozoa at 40 × 10^6/ml were aliquoted into tubes and treated with varying doses of 4HNE (0-400 µM) at 37^{o}C for 1 h. They were then washed with medium BWW and resuspended in a reaction mixture comprising 700 µl spermatozoa, 100 µl sodium azide (40 mM stock), 100 µl sodium succinate (50 mM stock) and 100 µl DCIP (500 µM stock). The cells were then sonicated and the absorbance of the samples was read at 600 nm every 2.5 min for 20 min. Blanks were also recorded containing no cells or no DCIP and their readings subtracted from the sample values.

For cytochrome oxidase activity, spermatozoa at 40 × 10^6/ml were treated with varying doses of 4HNE (0-600 µM) at 37^{o}C for 2 h. The cells were then lysed (100 µl 1% n-dodecyl-β-D-maltoside, 50 mM Tris, pH 7.8) for 1 h at 4^{o}C with constant rotation. The sample was then centrifuged (10,000 g, 10 min) to remove insoluble particulate matter and the supernatant collected for analysis. Oxidation of reduced cytochrome c was measured at

550 nm upon addition of 100 µl cell lysate and following the decrease in absorbance for 20 min. A positive control of completely oxidized cytochrome c was included by omitting the cell lysate and adding 100 µl potassium ferricyanide (100 mM). Complete inhibition of cytochrome C oxidase activity was demonstrated with azide (4 mM).

Supplementary Figures

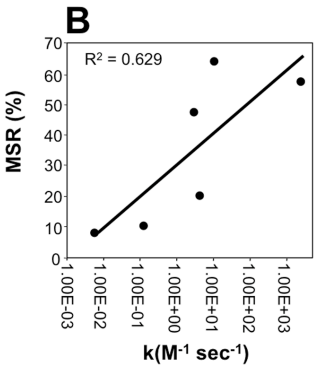


Supplemental Figure 1. Direct comparison of several electrophiles for their relative ability to stimulate mitochondrial ROS generation in populations if human spermatozoa at a dose of 100 µM after 2h incubation at 37°C. Data analyzed by ANOVA and values are presented as means ± SEM; ***$p < 0.001$; *$p < 0.05$ for differences with untreated control by Fisher's PLSD. All analyses replicated on 3 independent semen samples. Controls, open bars; treated, closed bars. ACR, acrylamide; IDA, iodoacetamide; BQ, 1,4 benzoquinone; 4HNE, 4-hydroxynonenal; EVK, ethyl vinyl ketone; NEM, N-ethylmaleimide; ACL, acrolein. Pos, positive control treated with 50 µM arachidonic acid.

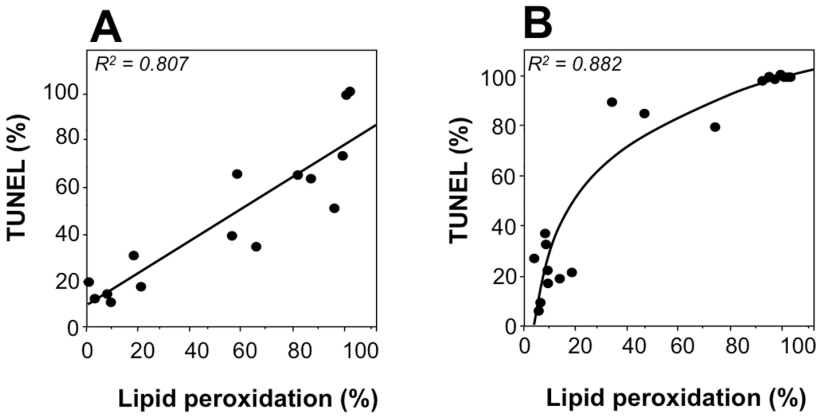
A

	Electrophilicity Index		Second order	
	ω HF	ω DFT	exp K (M^{-1} sec^{-1})	MSR
Acrolein	1.25	3.39	n/a	71.91
N-ethylmaleimide	1.93	5.16	1.03E+01	64.36
Ethyl vinyl ketone	1.19	3.22	2.44E+03	57.77
4-hydroxynonenol	0.29	0.91	2.86E+00	47.67
Benzoquinone	2.57	7.32	4.00E+00	20.30
Iodoacetamide	0.63	1.46	1.25E-01	10.31
Acrylamide	1.05	2.50	5.82E-03	8.01

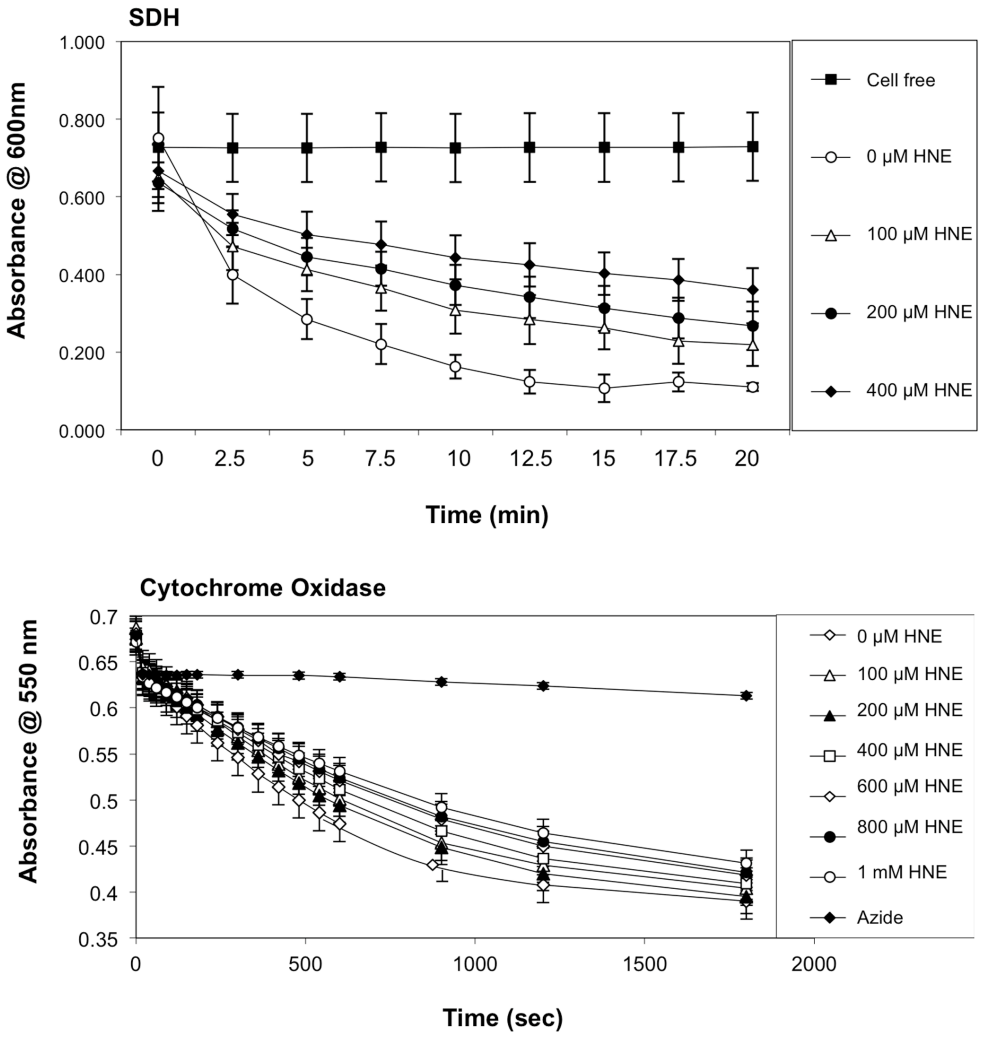
B



Supplemental Figure 2. Correlation between various measures of electrophilicity and the generation of mitochondrial ROS. measured using MSR following the exposure to a range of electrophiles for 2h at 37°C. (A) a summary of the calculations of electrophilicity based on theoretical considerations and (B) a plot of the experimental measurement of electrophilicity against mitochondrial ROS generation using reduced glutathione as the nucleophile.



Supplemental Figure 3. Relationship between DNA fragmentation as measured by the TUNEL assay and lipid peroxidation as measured using BODIPY C_{11}. (A) linear correlation with 4HNE (B) exponential correlation with acrolein.



Supplemental Figure 4 Biochemical assessment of succinic acid dehydrogenase (SDH) activity and cytochrome oxidase activity following the exposure of human spermatozoa to 4HNE. For these analyses spermatozoa at 40×10^6/ml were treated with the indicated doses of 4HNE at 37°C for 1 h. The cells were then washed and assayed for enzyme activity as described in Experimental Procedures. Only SDH activity was effectively suppressed by 4HNE treatment in keeping with the data presented in Fig. 7 indicating that SDH is a major target for 4HNE adduction in human spermatozoa.