Apoptosis Signal Transduction In Ejaculated Human Spermatozoa Following Physiological And Pathological Stimuli

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Objective: The presence of Fas (CD95) and caspase (CP) 8 activation (type I apoptosis), and CP-9 activation (type II apoptosis), along with the activation of their shared effector CP3 and CP1 have been reported in human spermatozoa. The objective of our study was to examine the role of receptor (type I) and mitochondrial (type II) as well as CP1 derived apoptosis signaling in ejaculated human spermatozoa in response to type I and II apoptosis inducers (Fas, CD95 and Betulinic acid, BA), oxidative stress (hypochlorous acid, HOCl) and capacitation.

Design: Prospective-controlled study.

Materials and Methods: Semen specimens collected from 15 healthy donors were separated into 7 aliquots. Two aliquots were incubated with 2 µg/mL CD95 antibody and 60 µg/mL Betulinic acid. Another aliquot was subjected to incubation with HOCl (10^3 mol/L) and incubated for 1h. The remaining pellet was re-suspended in 1 mL of BWW + 3% BSA at 5% CO₂ to induce capacitation for 3h. Controls were incubated under identical conditions with equal volumes of PBS. Using carboxyfluorescein derivatives, the levels of active caspase 1, 3, 8 and 9 were estimated with flow cytometry.

Results: Both CD95 and HOCl treatment did not activate any of the measured caspases. BA resulted in a significant increase in activation of CP9 and CP3 compared with controls (43.6 ± 13.4 vs. 25.5 ± 10.7, 45.3 ± 17.8 vs. 26.1 ± 8.2; P < 0.001). Following capacitation, compared with controls, a significant increase was seen in CP1 (44.1 ± 16.1 vs. 36.1 ± 12.2, P<0.05); CP9 (31.3 ± 15.0 vs. 21.3 ± 12.1, P<0.05) and CP3 activation (39.9 ± 17.6 vs. 28.9 ± 12.4; P < 0.05).

Conclusion: Ejaculated human spermatozoa may not display the complete signaling pathways in response to both physiological and pathological apoptotic stimuli. Mitochondria derived type II pathway appears to be predominant pathway in both stimuli and therefore should be the target of intervention.

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