Pathogenesis of Spermatozoal Apoptosis In Response to Anti-Cancer Treatment With Betulinic Acid

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Objective: Betulinic acid (BA) is a pro-apoptotic signal-transducing molecule that acts on mitochondria. It is experimentally used in the treatment of certain cancers. In human spermatozoa, the mitochondria-mediated apoptotic pathway involves activation of both the initiator caspase-9 as well as the effector caspase-3 while caspase-8 is involved in membrane receptor-mediated apoptosis. The objective of our study was to investigate the role of caspases in the pathogenesis of BA-induced apoptosis in human spermatozoa.

Design: Prospective-controlled study.

Materials and Methods: A total of 15 ejaculates from 15 healthy donors were collected after a sexual abstinence of 2 to 3 days. Unprocessed (neat) semen samples were washed in phosphate buffered saline (PBS) by centrifugation at 400 g for 5 min. The supernatant was discarded and the pellet was diluted in 1 mL PBS. Spermatozoa were incubated with BA (60 µg/mL) for 10 min at room temperature. In controls BA was replaced with PBS. Activated caspase-1, 3, 8, and 9 were determined using carboxyfluorescein derivatives and flow cytometry.

Results: Apoptosis was induced in samples incubated with BA compared to controls aliquots: activated caspase-9 (43.6 ± 13.4 vs. 25.5 ± 10.7, P < 0.001) and caspase-3 (45.3 ± 17.8 vs. 26.1 ± 8.2, P < 0.001). A significant increase was seen in the number of caspase-8 positive spermatozoa (46.3 ± 16.6 vs. 34.2 ± 16.4, P < 0.05), while caspase-1 was not significantly activated.

Conclusions: BA significantly triggers caspase-9 and caspase-3 activation in human spermatozoa. Caspase-8 activation may be a result of caspase ‘cross talk’. Our results indicate that spermatozoal mitochondria are extremely susceptible to specific agonists of apoptosis such as BA. Therefore the potential side effects of BA on the male reproductive system should be carefully examined.

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