P-061 Protective effect of melatonin against bleomycin, etoposide, and cisplatin (BEP) chemotherapy-induced testicular toxicity in Wistar rats: A biochemical, immunohistochemical and apoptotic genes based evidence

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Study question: Does exogenous melatonin (MLT) attenuate BEP-induced damage in testicular cells and spermatogenesis in a dose-dependent manner?
Summary answer: Melatonin protected the testes against BEP-induced testis damage through ameliorating nitro-oxidative stress, apoptosis, and inflammation. However, there was no significant difference between melatonin-treated groups.
What is known already: Recently, the prevalence of testicular cancer (TC), accounting for the most common cancer among young people of reproductive age (15–40 years), has risen internationally. BEP chemotherapy has increased the 5-year survival rate of TC patients at all stages of testicular germ cell tumors to 90-95%. However, BEP creates a high incidence of male infertility and even long-term genotoxic effects, which emerges as a critical health issue. Melatonin is a well-known potent antioxidant with widespread clinical applications that recently has been giving increasing attention to its role in male sub/infertility.
Study design, size, duration: 60 Adult male Wistar rats were randomly assigned to six groups (n=10/group). Group 1, 3, and 4 were injected with vehicle, 10 and 20 mg/kg of melatonin, respectively. Other groups received one cycle of bleomycin, etoposide, and cisplatin for a total of 3 weeks with or without melatonin. Melatonin administration started daily one week before BEP initiation continued on days 2, 9, and 16; and one week after the completion of the BEP cycle.
Participants/materials, setting, methods: Bodyweight, testes weight, Sperm parameters (count, motility, viability, and morphology), testosterone hormone level, testicular histopathology, stereological parameters, testicular level of malondialdehyde (MDA), nitric oxide (NO), and total antioxidant capacity (TAC), the expression of Bcl-2, Bax, Caspase-3, p53, and TNF-α(Real-time PCR and immunohistochemistry) were evaluated at the end of the study (day 35).
Main results and the role of chance: Our findings showed that melatonin restores the BEP-induced reduction in the body and testes weight ($P<.05$). the evaluation of quantitative analysis of the testes stereological procedures, QRT-PCR examination and immunohistochemical (IHC) staining revealed that melatonin reverses the BEP-induced impaired spermatogenesis ($P<.05$). Furthermore, melatonin rectifies BEP-induced disturbance on sperm count, motility, viability, and morphology. The testosterone level in the BEP-treated group was decreased significantly by comparison with the control group ($P<.01$). By contrast, co-administration of 10 and 20 mg/kg of melatonin could enhance the serum testosterone level significantly ($P<.05$). Moreover, melatonin enhanced the antioxidant status of the testis by elevating TAC and ameliorating MDA and NO levels. More notably, QRT-PCR examination indicated that melatonin therapy suppressed BEP-induced apoptosis by modulating apoptosis-associated genes such as Bcl-2, Bax, Caspase-3, p53 in the testis ($P<.01$). Besides, Co-administration of 10 and 20 mg/kg of melatonin with BEP regimen decreased significantly the population of p53 (54.21 ±6.18 % and 51.83±8.45, respectively) and TNF-αpositive cells (42.91±9.92 % and 33.57±2.97, respectively) by comparison to the BEP group.

Also, melatonin with low and high doses could enhance the expression of Bcl-2 protein in spermatogenic cells line (59.19±10.18 %, 63.08±5.23, respectively) compared to the BEP-treated group.

Limitations, reasons for caution: Owing to limited laboratory facilities we were not able to perform further studies to verify the mechanism of melatonin in the specific targets by using transfection technique and transgenic.

Wider implications of the findings: These findings can draw attention to the clinical application of melatonin and also suggest that melatonin may be an attractive agent for attenuating chemotherapy-associated male sub/infertility. This indolamine also may shorten the fertility recovery period in patients undergoing chemotherapy with the BEP regimen.

Trial registration number: N/A