Title: AZATHIOPRINE FAILS TO ALTER THE DOSE-RESPONSE CURVE OF d-TUBOCURARINE

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Introduction: Azathioprine (AZA) is a purine derivative structurally related to xanthines such as theophylline.[1] Clinical reports have documented reversal of the effect of nondepolarizing relaxants with both AZA and theophylline. Cyclic 3':5' adenosine monophosphate (cAMP)-mediated facilitation of neuromuscular transmission was implicated in the reversal. The importance of this interaction in terms of the magnitude of the shift in the dose-response curves to nondepolarizing relaxants has not been systematically studied. Because of the extensive use of AZA in both transplant patients and patients with myasthenia gravis, in this study we examined the importance of AZA interaction with d-tubocurarine (dTC) and studied the changes in cAMP in skeletal muscle.

Methods: The experiments were conducted according to NIH and institutional guidelines. Sprague-Dawley rats were anesthetized with I.P. pentobarbital, 60 mg/kg. After tracheostomy, the animals were ventilated with room air using a Harvard respirator to maintain physiologic venous blood gases. Three groups of animals were studied: controls (n=12), low-dose AZA (5 mg/kg, n=8), and high-dose AZA (50 mg/kg, n=8). After immobilization, the left gastrocnemius muscle was isolated and the tendon connected to a Grass FT03 force transducer. Baseline muscle tension was 50 g. Supramaximal pulses 0.15 Hz and 0.2 ms were applied to the sciatic nerve. The twitch tension was recorded. Following a stable twitch tension, 1 ml saline (control group) or AZA in 1 ml saline was administered IV. Five minutes later incremental doses of dTC were administered to achieve 90-95% twitch inhibition.

During an infusion of dTC while maintaining a steady twitch depression of 25% of control for over 10 minutes, the response to 50 mg/kg bolus of AZA administered via contralateral vein was recorded (n=3). Following all the twitch studies, a sample of gastrocnemius muscle from the contralateral limb was taken for cAMP analysis, using ^{125}I-radioimmunoassay.[2]

Dose-response curves were plotted on a log-probit scale and the effective dose (ED) values were calculated. The significance of the data was tested by the student t-test and analysis of variance with values of $p < .05$ being significant.

Results: There was no significant shift in the dose-response of dTC by AZA (see Table). High-doses of AZA administered during steady state twitch depression produced an increase in twitch from 25 to 50% within a minute, which was maintained for three minutes and reverted to original levels in 5 to 10 minutes. The cAMP level in control muscle was 120 + 18 pmol/mg protein. Low-dose AZA did not cause a further increase in cAMP (163 ± 24 pmol/mg protein). High-dose AZA, however, caused a significant increase in cAMP (levels 340 ± 49, $p < .002$) compared to the control or low-dose AZA group.

Table: ED for dTC with/without AZA (Means ± SE)

	ED_{50}	ED_{95}
Control	0.058 ± 0.006	0.117 ± 0.009
5 mg/kg AZA	0.061 ± 0.002	0.132 ± 0.010
50 mg/kg AZA	0.061 ± 0.006	0.127 ± 0.013

Discussion: This study documents that AZA administered five minutes prior to dTC in therapeutic (5 mg/kg) or pharmacologic (50 mg/kg) doses does not cause a significant shift in the dose-response curve of dTC. Pharmacologic doses of AZA produced a transient reversal of twitch height during steady state twitch depression obtained with continuous infusion of dTC. Whether therapeutic doses of AZA also cause this transient effect is unknown. Increases in cAMP were implicated as the cause in the reversal of nondepolarizers.[1] This study documents that even with 10 times the therapeutic dose of AZA, there was only a three-fold increase in cAMP with no significant shift in the ED values of dTC. This is consistent with theophylline-dTC interaction studies where toxic concentrations of theophylline caused no shift in ED values, while therapeutic concentrations in fact potentiated dTC. In conclusion, acute administration of AZA does not cause a shift in dose-response curves of dTC.

References

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