Azathioprine Fails to Alter the Dose-response Curve of d-Tubocurarine in Rats

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Cyclic 3′5′ adenosine monophosphate (cAMP) mediated facilitation of neuromuscular (NM) transmission was previously implicated in the mechanisms of the reversal of nondepolarizing NM relaxants by azathioprine (AZA). This interaction of d-tubocurarine (dTC) with AZA was re-examined in rats and correlated to changes in cAMP in the same muscle. Three groups of animals were studied: controls, low-dose AZA (5 mg/kg), and high-dose AZA (50 mg/kg). After AZA or saline administration, dose-response (DR) curves for inhibition of gastrocnemius twitch tension by dTC were constructed. Contralateral gastrocnemius muscle was sampled for cAMP levels. In another group of animals, the response to 5- and 56-mg/kg boluses of AZA was recorded during a steady-state twitch depression maintained with an infusion of dTC. No significant shift in the DR curve of dTC was observed following low- and high-dose AZA. During steady-state twitch depression, high-dose AZA, however, caused a transient reversal of twitch lasting 5–10 min. High-dose AZA caused a significant (P < 0.0006) elevation of cAMP levels (340 ± 49 pmol/mg prot) compared to control (120 ± 18) and low-dose (163 ± 24) AZA groups. These studies, therefore, document transient reversal of twitch tension by 50 mg/kg doses of AZA during a steady-state dTC infusion. On the other hand, AZA administered prior to dTC in low (5 mg/kg) and high (50 mg/kg) doses failed to cause a significant shift in the dTC DR curve. A three-fold increase in skeletal muscle cAMP induced by high-dose AZA does not alter dTC DR curves. (Key words: Antagonists, neuromuscular relaxants: azathioprine. Metabolism: cyclic 3′5′ adenosine monophosphate. Neuromuscular relaxants: d-tubocurarine.)

AZATHIOPRINE (AZA), a thiopurine compound which acts as a purine antagonist, is widely used today as an immunosuppressive agent for organ transplantation, in the treatment of patients with myasthenia gravis, and other autoimmune disorders. Therefore, numerous surgical patients receive AZA in the perioperative period. Dretchen et al. reported that AZA can reverse the effects of nondepolarizing neuromuscular (NM) relaxants in humans. Their findings were confirmed in the cat when reversal of a single dose of d-tubocurarine (dTC) was observed during intra-arterial injection of AZA. The proposed mechanism for this drug interaction was related to the structural resemblance of AZA to theophylline and, therefore, to its alleged ability to act as a phosphodiesterase inhibitor. Although cyclic 3′5′ adenosine monophosphate (cAMP) levels were not measured at the nerve terminal or NM junction, it was suggested that the resulting increase in cAMP levels enhanced the release of acetylcholine at the NM junction, thus facilitating NM transmission.

In this study, we examined the dTC-AZA interaction in rats as it pertains to: 1) the magnitude and shift in dTC dose-response curves, 2) the duration of effect, and 3) significance of changes in skeletal muscle cAMP levels.

Materials and Methods

Twitch Studies

The experiments were conducted according to NIH and institutional guidelines. Age-matched Sprague-Dawley rats were anesthetized with intraperitoneal pentobarbital (60 mg/kg), and the gastrocnemius preparation was isolated as previously described.

Three groups of animals were studied: controls (n = 8); low-dose AZA (5 mg/kg, n = 8), which is the clinically administered dose; and high-dose AZA (50 mg/kg, n = 8). Following stabilization of the baseline twitch tension for at least 10 min, 1 ml saline (or AZA in 1 ml saline) was administered intravenously. Five minutes later, incremental doses of dTC were administered to achieve 90–95% twitch inhibition. The time interval to recovery from 25% to 50% of the control twitch response was also recorded.

In another set of experiments during an infusion of
TABLE I. Neuromuscular Pharmacodynamics of dTC with/without Azathioprine

<table>
<thead>
<tr>
<th></th>
<th>ED50 (mg/kg)</th>
<th>ED90 (mg/kg)</th>
<th>Rec. Time (Min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.058 ± 0.006</td>
<td>0.117 ± 0.009</td>
<td>4.9 ± 1.0</td>
</tr>
<tr>
<td>5 mg/kg AZA</td>
<td>0.061 ± 0.002</td>
<td>0.132 ± 0.110</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td>50 mg/kg AZA</td>
<td>0.061 ± 0.006</td>
<td>0.127 ± 0.013</td>
<td>4.4 ± 3.7</td>
</tr>
</tbody>
</table>

Data given as mean ± SE.

dTC, while maintaining a steady-state twitch depression of approximately 25% of control for over 10 min, the response to a 5 mg/kg AZA bolus (n = 3) and a 50 mg/kg AZA bolus (n = 3), administered via the contralateral internal jugular vein, was recorded.

CAMP Determinations

Following twitch studies, a sample of gastrocnemius muscle from the contralateral limb was taken for CAMP analysis and immediately frozen at −70°C. The time elapsed between AZA administration and muscle sampling was approximately 45–60 min. Levels of CAMP in gastrocnemius muscle were measured using 125I-radioimmunoassay (RIA) following previously described methods.⁶⁷ Levels of CAMP were expressed as picomole/milligram protein (pmol/mg prot) in muscle.

Statistical Analysis

Dose-response curves were plotted on a log-probit scale and the effective dose (ED) values were calculated.⁸ The significance of the data was tested by analysis of variance with values of P < 0.05 being significant.

Results

Initial administration of saline or AZA without dTC at either dose did not alter baseline twitch tension. AZA, at 5 mg/kg and 50 mg/kg, failed to evoke a significant shift in the dose-response curve of dTC (table 1). In addition, there was no significant difference in the time required for recovery of paralysis from 25% to 50% of control of twitch height. High-dose (50 mg/kg) AZA administered during steady-state dTC infusion produced an increase in twitch tension from 25% to approximately 50% of control within 1 min. This effect was maintained for about 3 min and reverted to baseline levels within 5–10 min (fig. 1).

The CAMP level in gastrocnemius muscle of control animals was 120 ± 18 pmol/mg prot (fig. 2). Low-dose AZA did not induce a further increase in CAMP (163 ± 24 pmol/mg prot). High-dose AZA, however, caused a three-fold increase in CAMP levels (340 ± pmol/mg prot, P < 0.006) compared to control or low-dose AZA groups.

Discussion

This study documents that low (5 mg/kg) and high (50 mg/kg) doses of AZA administered 5 min prior to dTC: 1) do not significantly alter the dose-response curve to dTC; 2) evoke no significant changes in twitch recovery times; and 3) cause a three-fold increase in CAMP levels in the contralateral but same muscle despite the lack of interaction between AZA and dTC. These findings suggest that this magnitude of change in CAMP in muscle has no effect on NM transmission.

During steady-state partial NM blockade achieved by a continuous infusion of dTC, the extent of block was transiently and incompletely reversed by high-dose AZA; low-dose AZA had no recordable effect. The rapid onset and transient nature of the effect of high-dose AZA suggests that mechanisms other than phosphodiesterase inhibition are involved. Although AZA is rapidly cleaved to 6-mercaptopurine in vivo,¹ the transient and incomplete reversal of block observed may be related to CAMP modulation of the NM junction at different intracellular sites. A 5-min period preceded the initiation of the dTC dose-response curve, so that the AZA effect, however transient, would be expressed in its entirety. The brief duration of high-dose AZA on
Azathioprine Effects on cAMP in Muscle

![Azathioprine Effects on cAMP in Muscle](image)

**Fig. 2.** Azathioprine effects on gastrocnemius cAMP levels. Therapeutic doses of AZA cause an insignificant rise in cAMP levels. High doses, however, cause a significant (*P < 0.006*) three-fold increase in cAMP levels.

twitch strength may be explained by the pharmacokinetics of AZA. Subsequent to intravenous infusion in humans and the rapid conversion to 6-mercaptopurine in plasma AZA catabolites are taken up by cells, leading to a very rapid fall in blood levels and removal from any extracellular sites of action. The observation that low-dose AZA has no effect during continuous infusion of dTC is consistent with the results of Chapple et al. They reported no effect of boluses of 1 or 5 mg/kg of AZA on cat gastrocnemius twitch after partial paralysis with atracurium, another nondepolarizing muscle relaxant. More recently, a study in humans has, in fact, confirmed the negligible clinical effect of AZA on twitch depression produced by atracurium, vecuronium, and pancuronium.

The mechanism of the transient reversal of AZA on twitch response could not be characterized by the present studies. Nevertheless, several reports suggest that cAMP modulates varying sites of the in vivo twitch response. These would include altered NM transmission as it pertains to pre- and post-ganglionic acetylcholine release, the expression of desensitization at the acetylcholine receptor, and fundamental aerobic processes. Because the molecular skeleton of AZA resembles theophylline, the former may facilitate the release of catecholamines, which are known to enhance NM transmission. Certainly, increased circulating catecholamines could also account for the increased muscle cAMP levels found in our study 45-60 min post-AZA administration. Catecholamines are known to be potent activators of adenylate cyclase. Nevertheless, the elevated cAMP levels in muscle probably have nothing to do with the antircure effect.

In conclusion, AZA produces a brief antagonism of NM blockade in the in vivo rat model at high (pharmacological), but not at low (clinical), doses when administered under steady-state partial neuromuscular blockade. The brevity of effect is probably due to rapid plasma clearance of AZA, which would also tend to limit the clinical significance of this effect in humans as well. Prior administration of AZA in low or high doses does not shift the dose-response curves of dTC. Skeletal muscle levels of cAMP are elevated in the high-dose AZA group, but this three-fold change does not appear to influence the dose-response curves of dTC.

**References**

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