Effect of Quinine on the Morphology of Mouse Testes

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Single injection of quinine in a maximum tolerable dose to BALB/c mice caused morphological changes in the testes and suppressed spermatogenesis. Gonocytes in all layers of the spermatogenic epithelium, interstitial endocrinocytes, and sustenocytes proved to be sensitive to the toxic effect of the drug. The majority of detected morphological changes were reversible.

Key Words: *mice; testes; quinine*

Quinine, an alkaloid from cinchona bark, is used for the treatment of chloroquine-resistant malaria [4], in obstetrics [5], and for arresting muscle convulsion [1]. Quinine causes nausea, vomiting, allergic reactions, suppresses the CNS, and exerts toxic effects on the gastrointestinal system and blood [4]. Though this drug has long been used in medicine, its toxic effects still attract much attention. Hematotoxic effects of quinine have been recently discovered [10]. It induces uremic syndrome [1]. Increased incidence of malaria observed in recent years [6] makes investigations of quinine a problem of special importance. Moreover, synthetic quinine derivatives are now widely used in cardiology [3] and oncology [11].

We examined the testes of mice during early terms after quinine injection.

MATERIALS AND METHODS

Experiments were carried out on 70 male BALB/c mice (20-25 g) from Rassvet Breeding Center, Tomsk. The animals were kept under standard vivarium conditions with a day-night light regimen in standard plastic cages with small wood chips, no more than 15 animals per cage, at 20-22°C and 50% humidity. Gas exchange volume was 8:10. Mice were fed standard PK 120-3 fodder. Quinine hydrochloride was injected intraperitoneally in a single maximum tolerable dose (300 mg/kg) determined by graphic probit-analysis after 30-day observations [2]. Controls were injected with an equivalent volume of the solvent. Mice were sacrificed by cervical dislocation 6 h and 2, 4, 6, 15, and 20 days after injection of quinine, 5 animals per point. The testes were removed, fixed in Carnoy fluid, and 5-μ paraffin sections were stained with hematoxylin and eosin. Morphological analysis included estimation of spermatogenesis index, quantitation of normal spermatogonias, tubules with meiosis stage 12, tubules with desquamated epithelium interstitial endocrinocytes [7,8], and of the total number of spermatozoa (TNS) in the epididymis. The cells were counted using a Goryaev's chamber [7]. The significance of differences was evaluated by the Wilcoxon—Mann—Whitney test.

RESULTS

Morphological analysis of the testes showed spermatozoon karyorhexis and karyopyknosis in the presence of slight interstitial edema in some convoluted tubules as early as 6 h after injection of quinine. Dead gonocytes were desquamated into the tubular lumen (Fig. 1). The interface between the cells of the spermatogenic epithelium (SE) was sometimes unclear, the cytoplasm of these cells was opaque. Giant mono-, bi-, or polynuclear spermatogenic cells, some of them with fragmented and pyknotic nuclei, were seen in some convoluted tubules during this period and later after injection of the drug (Fig. 2). On days 2-4 postinjec-

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tion vascular changes became more pronounced, basal membrane of convoluted tubules was often destroyed, some of them had foci of destroyed SE. Spermatogonias with karyopyknosis and karyorhexis were often seen. On day 6 postinjection morphological changes were less expressed. SE looked thinned some convoluted tubules. Moreover, supporting cells virtually disappeared from some tubules. By days 15-20 after injection of quinine, the morphological picture of testes in experimental mice was virtually the same as in the control, with just slight interstitial edema in the gonads of experimental animals.

On day 6 of the experiment, index of spermatogenesis decreased significantly in mice treated with quinine in comparison with the control (Table 1), which indicated thinning of SE. On days 2-4 of the experiment, the number of normal spermatogonias decreased significantly, cell population decreased by 49-53%. Later this parameter recovered and returned to the control values. The number of tubules with desquamated epithelium sharply increased as soon as 6 h after injection of quinine, indicating a direct toxic effect of the drug on gonocytes. Intense desquamation of SE cells was observed for 4 days, which could be due to appearance of pathologically changed cells subjected to elimination under the effect of the drug. Later this parameter did not differ from the control (Table 1). Quinine did not suppress meiotic activity of gonocytes, but significantly increased this parameter on day 2 of the experiment. Interstitial endocrinocytes were sensitive to toxic effect of quinine. The count of Leidig cells decreased by 40% 6 h after injection of quinine in comparison with the control. In later terms this parameter did not differ significantly from the control. Regeneration of interstitial endocrinocytes is stimulated by a local intertesticular factor produced by Sertoli cells [9], and therefore recovery of Leidig cell population 2 days after injection of quinine is an indirect proof of retained functional activity of sustentocytes, though, as we mentioned before, quinine exerted a toxic effect on these cells.

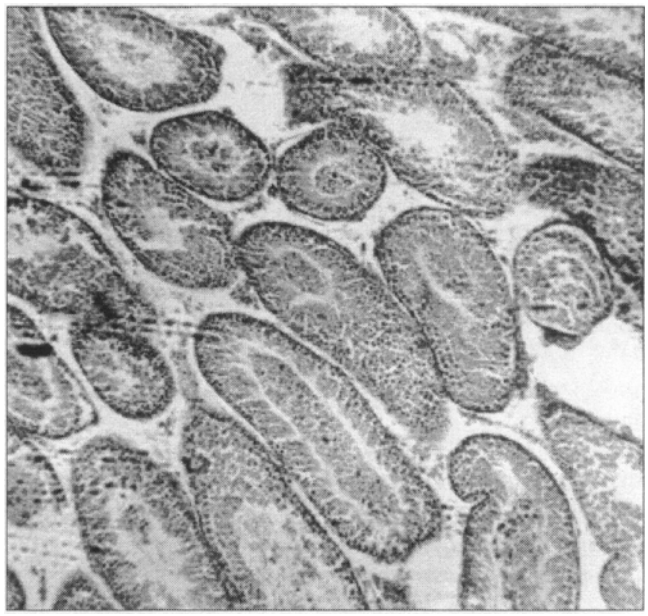

Fig. 1. Mouse testis 6 h after single injection of quinine in a maximum tolerable dose. Intense desquamation of epithelial cells into the lumen of convoluted tubule. Hematoxylin and eosin staining, ×90.

Quantitation of TNS in the epididymis of mice injected with quinine showed oligospermia during the first 2 days of the experiment. This parameter remained below the norm during the subsequent 2 weeks, but the differences were insignificant. On day 20 after quinine injection TNS decreased again (70% of the

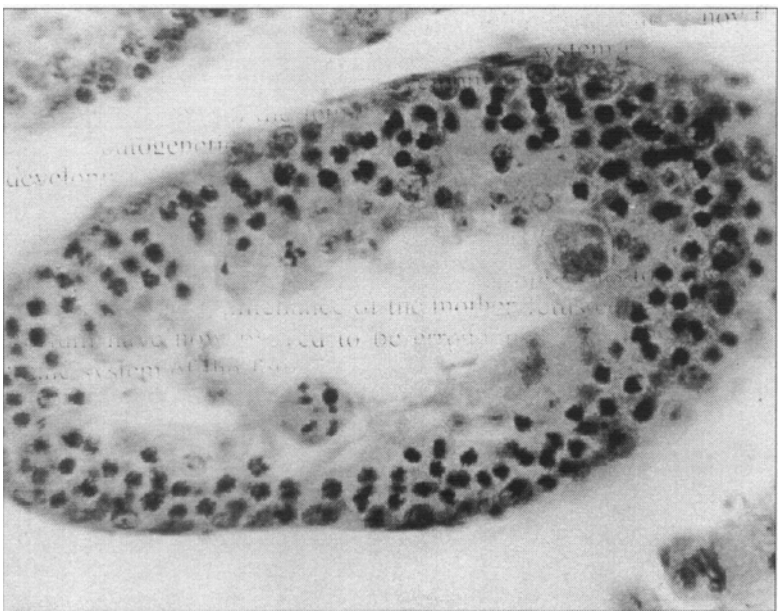

Fig. 2. Convoluted seminal tubule on day 6 after a single injection of quinine in the maximum tolerable dose. Giant multinuclear cells, one with pyknotic and fragmented nucleus, ×400.

TABLE 1. Morphological Parameters of Generative and Endocrine Function of Mouse Testes after Single Injection of 300 mg/kg Quinine ($X \pm m$)

Parameter	Control	Time postinjection					
		6 h	2 days	4 days	6 days	15 days	20 days
Spermatogenesis index, arb. units	3.15±0.06	3.35±0.13	3.44±0.08	3.29±0.12	2.81±0.03*	3.15±0.04	3.31±0.06
Number of normal spermatogonias	15.32±1.05	14.25±1.42	7.77±0.62*	7.09±0.67*	10.22±0.73	10.56±1.17	17.35±0.92
Tubules with desquamated epithelium, %	0.40±0.24	6.75±2.53*	0.80±0.58	8.00±2.68*	3.00±2.68	0.75±0.48	0.75±0.25
Tubules with meiosis stage 12, %	1.00±0.55	0.50±0.29	4.50±0.96*	1.25±0.25	0.50±0.29	0.75±0.25	3.00±0.71
Relative number of Leidig cells	9.84±0.54	5.78±0.49*	10.96±0.72	9.24±0.79	9.38±1.34	11.33±2.53	9.79±1.48
Total count of spermatozoa, 10^6	3.03±0.37	1.96±0.45*	2.13±0.30*	2.42±0.35	2.33±0.26	2.23±0.22	2.1±0.4*

Note. *$p<0.05$ *vs.* the control.

control). Taking into account the duration of spermatogenesis stages in mice [7] we conclude that oligospermia observed at the end of the experiment indicates damaging effect of quinine on spermatides.

Hence, single injection of quinine in a maximum tolerable dose to BALB/c mice caused disorders in the testicular morphology and suppressed spermatogenesis. Gonocytes of all SE layers, interstitial endocrinocytes, and sustentocytes were sensitive to the toxic effect of the drug. The majority of these disorders were reversible.

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