

## ANTISPERMATOGENIC ACTIVITY OF GOSSYPOL IN RATS FED ON LOW-POTASSIUM DIET

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**ABSTRACT** Adult male rats were fed on either low-K or normo-K diet for 3 months. Gossypol acetic acid was given po to all rats in a daily dose of 15 mg/kg, 6 d/wk for the same period of time. At the end of the regimen, plasma K<sup>+</sup> concentration was determined and testicular histology examined. The plasma K<sup>+</sup>

concentration of the rats fed on low-K diet was far lower than both the reported normal value and the value of the rats fed on normo-K diet, indicating a K deficient status of the former. Both the extent and severity of damage of seminiferous epithelia were significantly more pronounced in low-K fed than in normo-K fed rats. It is concluded that the antispermatogenic activity of gossypol can be enhanced by K deficiency, a contributing factor in the development of gossypol-induced sterility.

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Received 1982 July 15      Revised 1982 Oct 22

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**KEY WORDS** gossypol; potassium deficiency; male antifertility agents; antispermatogenic agents

Although gossypol, a new antifertility agent discovered by Chinese scientists, has been used in about 9000 men in China<sup>(1,2)</sup> and quite a few abroad<sup>(3)</sup>, several problems, including irreversibility of fertility in a small percentage of volunteers, preclude its expanded use as an official male antifertility agent at present.

The level of K intake could modify the effect of gossypol on the intracellular K<sup>+</sup> concentration<sup>(4)</sup> and Na-K-ATPase activity<sup>(5,6)</sup> in rats or guinea pigs; it also influenced the incidence of hypokalemic paralysis among subjects taking gossypol<sup>(7,8)</sup>. These data point to a mechanism for the gossypol effect on K metabolism dependent upon dietary K level. It seems tempting to postulate that dietary K level might be implicated in the antispermatogenic activity of gossypol. The present study was undertaken in the hope to clarify this problem.

## METHODS AND RESULTS

18 adult ♂ rats, weighing  $285 \pm$  (SD) 15 g, were divided at random into 2 groups. One group was fed on a low-K (6 mmol/kg) diet and the other group on a normo-k (146 mmol/kg) diet for 3 months. The feeds were prepared according to Zheng et al<sup>(9)</sup>. Rats were allowed free access to laboratory chow and tap water. All the rats were given intragastrically gossypol acetic acid (in 1% CMC) 15 mg/kg daily, 6 d/wk, for the same period of time. Gossypol acetic acid was supplied by the Experimental workshop of this Institute (batch number 791203, purity 101%).

At the end of the regimen, the rats were anesthetized with Na pentobarbital ip and blood samples were withdrawn from the abdominal aorta for the flame photometric determination of plasma K<sup>+</sup> concentration. Testes were examined microscopically after fixation and H-E staining. The damage of seminiferous epithelia was determined by a semi-quantitative method

<sup>(10)</sup> with minor modifications. In brief, 50-100 tubular sections were examined and the % of sections with significant cellular damage served as the index for extent of damage; the severity was estimated mainly on the presence of atrophic and calcified tubules. The results are shown in Table 1.

Tab 1. Gossypol-induced testicular damage ( $\bar{x} \pm$  SD)

Dietary regimen	Rats	Plasma K <sup>+</sup> (mmol/l)	Seminiferous tubular damage	
			Extent (%)	Severity
Low-K	9	$2.1 \pm 0.3^{***}$	$55 \pm 23^{**}$	+++
Normo-K	9	$3.6 \pm 0.3^{***}$	$29 \pm 24^{**}$	+

\*\*P<0.05, \*\*\*P<0.001

The plasma K<sup>+</sup> concentration of rats fed on low-K diet was significantly lower than that of the normo-K fed rats, and was much below the reported values for normal rats<sup>(9,11)</sup>, indicating a K-deficient status of low-K fed rats. In these rats, both the extent and severity of damage of the seminiferous epithelia were far more pronounced than in the rats fed on normo-K diet. Results showed that the antispermatogenic activity of gossypol was enhanced by K deficiency.

## DISCUSSION

In a few % of volunteers, fertility may not recover as long as 3-4 years after cessation of gossypol administration<sup>(2)</sup>. Apparently, irreversibility is the result of extensive damage of the seminiferous epithelia as shown by testicular biopsy. As to the reason why such a change only occurs in a few of the subjects taking gossypol, no satisfactory explanation has yet been suggested.

K contents of various food are widely different, for example, the content of washed rice is only 1/3 of that of standard flour<sup>(12)</sup>. Due to the diversity of food habit of men, the daily K intake of a person may range from as low as 23 or 26.9 mmol to 100 mmol or more<sup>(8,13)</sup>. As the present study pointed out that low-K intake could enhance the antispermatogenic

activity of gossypol, it appears highly probable that the degree of injury of the seminiferous epithelium induced by gossypol will be different in persons having different K intake, and in those having the lowest, long continued use of gossypol may lead to extensive tubular damage and irreversibility. Present study highlights the need of K supplementation in volunteers with insufficient dietary K level, as deficient K intake is not of uncommon occurrence both in China and abroad and has aroused increasing attention to the public<sup>(12)</sup>.

The problem, whether simple K deficiency can affect spermatogenesis, has not been settled yet. Preliminary work in this laboratory indicated a possible influence of K deficiency on spermatogenesis causing degeneration of a few germ cells. Yet it is far from conclusive as a fair % of these cells do show degenerative changes also in normal rats<sup>(14)</sup>. The clarification of this problem, which will need precise quantitation of testicular histology, would contribute to the elucidation of the mechanism of the enhancement effect of K deficiency on gossypol action. Further work is under way. However, present data can amply demonstrate the enhancement effect whatever the mechanism would be.

There must be factors other than K deficiency that might influence the antispermato-genic activity of gossypol as well, since individual variation of the degree of damage of seminiferous epithelia induced by gossypol is still quite marked in rats fed on the same kind of diet<sup>(15)</sup>.

**ACKNOWLEDGMENTS** We are grateful to WANG Yi-fei of the Shanghai Second Medical College for his help in histological examination, and to CAI Ming and PAN Yu-ying of this Institute for their technical assistance.

This work received financial support from the World Health Organization's Special Programme of Research, Development and Research Training in Human Reproduction.

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