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Some Non-hormonal Properties of 17β-Hydroxy-17α-methyl-5α-androstano[2,3-*c*]furazan (Furazabol)

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Furazabol showed non-specific inhibitory activities to the responses of guinea pig ileum to various spasmogens, and the activities were considerably higher than those of papaverine. It inhibited also the response of guinea pig *vas* deferens to adrenaline, depressed spontaneous movements of guinea pig atria and rat uterus and showed a slight and temporal fall in blood pressure which was not affected by the pretreatment of atropine. These data suggest that furazabol has a papaverine-like property. Furazabol showed serum cholesterol-lowering action in normal rats and cholesterol-fed chicks. In addition, it exerted curative action on gastric stress ulcer in rats.

17β-Hydroxy-17α-methyl-5α-androstano[2,3-*c*]furazan (furazabol) is an anabolic steroid which was synthesized by Shimizu, *et al.*[2] in 1965. According to Kasahara, *et al.*,[3,4] it is 3 times in anabolic activity and 29 times in nitrogen-retaining activity but rather weak in androgenic activity as compared with methyltestosterone under oral administration. In addition, this compound has been reported to possess other hormonal activities, *e.g.*, gonadotropin-inhibiting, estrus-inhibiting and progestational ones, though they are very weak. The present paper deals with some non-hormonal properties of this compound clarified in further pharmacological studies.

Material and Method

Material——Furazabol was used as solutions in 60% aqueous ethanol for the test with isolated organs, 90% aqueous propylene glycol for the blood pressure test and in vegetable oil for the other tests.

Isolated Organs——Experiments were done on the ileum, *vas* deferens and atria isolated from male guinea pigs weighing about 200 g. Each of these smooth muscle specimens was suspended in an organ bath containing 50 ml of Tyrode's solution to record the movement on a smoked paper. The bath temperature was maintained at 29—30° for the test with the ileum and *vas* deferens, and at 37—38° for the test with the atria and uterus. The physiological solution was bubbled with air, except for the solution for the atria test which was aerated with O_2 and CO_2 (95 : 5). Furazabol solution was added to the bath at a constant volume of 0.5 ml.

Blood Pressure, Respiration and Electrocardiogram——Male and female cats weighing 2.5—3.3 kg were anesthesized with urethane (1.5 g/kg, *s.c.*). Blood pressure at the carotid artery, respiration and electrocardiogram (lead I) were recorded on a polygraph and electrocardiograph, simultaneously.

Serum Cholesterol-lowering Action——Male Wistar rats weighing about 100 g were subjected to daily subcutaneous injections of furazabol for 9 days. The animals were sacrificed by bleeding from the carotid artery on the day after the last injection to measure serum cholesterol (Zak-Henly's method[5]).

Chicks weighing about 400 g were fed the chick chow diet enriched with 2% cholesterol and 5% sesame oil during the experiment. Blood samples were collected biweekly from the brachial vein to measure serum cholesterol. Furazabol was administered intramuscularly twice a week.

1) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
2) M. Shimizu, G. Ohta, K. Ueno, T. Takegoshi, Y. Oshima, A. Kasahara, T. Onodera, M. Mogi and H. Tachizawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 895 (1965).
3) A. Kasahara, T. Onodera, M. Mogi, Y. Oshima and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **13**, 1460(1965).
4) A. Kasahara, T. Onodera, H. Tachizawa, Y. Oshima and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **14**, 285 (1966).
5) A.A. Henly, *Analyst*, **82**, 286 (1957).

Curative Action on Experimental Gastric Ulcer——Experimental gastric ulcer was induced according to the method described by Takagi and Okabe.[6] Male Wistar rats weighing about 250 g were placed in restraining cages and immersed in a water bath (22—27°) for 20 hours. Starting two days after this treatment, furazabol was injected subcutaneously for 10 days. The animals were killed on the day after the last medication and the stomachs were removed to measure the areas of ulcerated lesions.

Result and Discussion

Papaverine-like Action

On the isolated guinea pig ileum, furazabol depressed the slightly raised tonus by solvent per se and inhibited the spasmodic response to different kinds of spasmogens (Fig. 1). The comparison was made of IC50 (the concentration of antagonist reducing the contractile effect of a standard dose of agonist by 50%) between furazabol and papaverine. As shown in Table I, furazabol was proved to be more potent in antispasmogenic activity than papaverine.

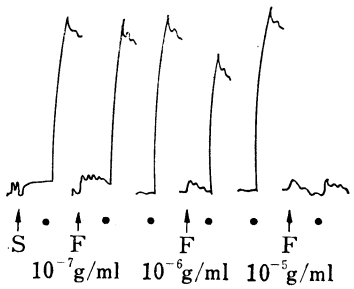

Fig. 1. Effect of Furazabol on the Response of Guinea Pig Ileum to Acetylcholine

S: solvent; F: furazabol; ●: acetylcholine, 10^{-8} g/ml

Furthermore, furazabol, contrary to atropine, clearly inhibited the intestinal contraction caused by a high concentration (10^{-4} g/ml) of acetylcholine. These data indicate that furazabol as well as papaverine antagonizes to acetylcholine non-competitively.

The effects on the other preparations are summarized in Table II. Furazabol produced a slight fall in cat blood pressure which was not affected by the pretreatment of atropine. The drug also inhibited spontaneous movements of the uterus and atria, and the contractile response of the *vas* deferens to adrenaline. Considering that the inhibiting effects were observed very rapidly, furazabol seems to combined with the smooth muscle cells readily.

TABLE I. Comparison of IC 50[a] between Furazabol and Papaverine

Compound	Spasmogens (g/ml)[b]				
	Ach, 10^{-7}	Hist, 10^{-6}	Nic, 10^{-6}	5-HT, 10^{-6}	$BaCl_2$, 10^{-4}
Furazabol	3×10^{-6}	1×10^{-5}	3×10^{-7}	2.5×10^{-6}	6×10^{-6}
Papaverine	3×10^{-5}	3.5×10^{-5}	8×10^{-6}	2.5×10^{-5}	1.5×10^{-5}

Three preparations were used for obtaining each IC50 value.
a) See the text.
b) Ach=acetylcholine; Hist=histamine; Nic=nicotine; 5-HT=serotonin

TABLE II. Effects of Furazabol on Various Preparations

Preparation	Dose of furazabol	Effect
Guinea pig *vas* deferens[a]	10^{-5} g/ml (pretreatment for 1min)	inhibition by about 65% of the response to adrenaline (10^{-6} g/ml)
Guinea pig atria[a]	10^{-5} g/ml	exertion of negative inotropic effect No influence on the positive inotropic response to adrenaline (10^{-8} g/ml)
Rat uterus[a] (diestrus stage)	5×10^{-6} g/ml	complete inhibition of spontaneous movement
Cat blood pressure[b]	0.5 mg/kg or more	causing a slight and temporal fall No influence by pretreatment of atropine (3 mg/kg)

Number of preparations used are 4 and 5 for *a*) and *b*), respectively.

6) K. Takagi and S. Okabe, *Jap. J. Pharmacol.*, **18**, 9 (1968).

As already known, various steroids antagonized to several spasmogens. Bass and Setliff[7] have reported that cortisone acetate shows antipilocarpine action, and Trethewie[8] has described that testosterone is antagonistic to histamine. The results obtained in the present study have revealed that furazabol has papaverine-like property on the smooth muscles.

Serum Cholesterol-lowering Action

Table III illustrates serum cholesterol levels in rats subjected to subcutaneous injections of furazabol for 9 days. Furazabol exhibited statistically significant reduction in serum cholesterol, but this action slightly weaker than that of triparanol, a cholesterol-lowering agent.

TABLE III. Serum Cholesterol-lowering Effect of Triparanol and Furazabol in Rats

Compound	Dose (mg/day)	No. of animals	Serum cholesterol (mg/100 ml)
Control	—	7	61.1±1.9[a]
Furazabol	0.2	6	64.4±2.4
	1.0	7	57.6±3.8
	5.0	7	54.0±2.1[b]
Triparanol	0.2	6	57.9±3.5
	1.0	7	48.5±3.2[b]
	5.0	7	40.1±1.5[b]

a) mean ±standard error
b) Significantly different from control ($p<0.05$, *t*-test).

Serum cholesterol levels in hypercholesterolemia chicks were not affected by injection of furazabol for 6 weeks, but slightly decreased by further continueing the injection. Mean cholesterol levels in the liver of the animals killed after 8 weeks were 82.6 and 71.6 mg/g in control and furazabol treated groups, respectively, being not significantly different each other. Therefore, it is apparent that the antihypercholesterolemic effect of furazabol is not due to the transfer of cholesterol from the blood to the liver.

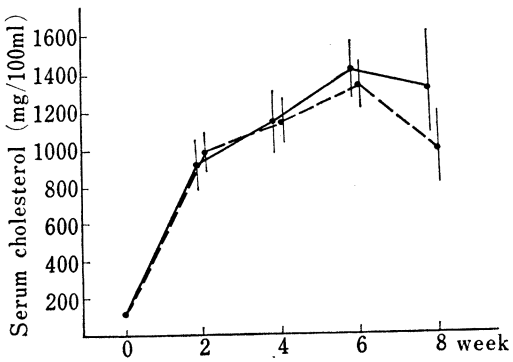

Fig. 2. Effect of Furazabol on Serum Cholesterol in Cholesterol Fed Chicks
——: control (sesame oil 0.5 ml twice a week)
——: furazabol (5 mg twice a week)
Each bar represents the standard error (5 chicks/group).

These findings differ from those cases of cholesterol-fed rabbits[9,10] in which furazabol markedly depressed the elevation of serum cholesterol. In addition, the oral chronic toxicity test[11] (six months) revealed that furazabol caused a 50% reduction of serum cholesterol in rats. This difference seems to be associated with animal species.

Anti-ulcer Action

Table IV shows the curative effect of furazabol on gastric stress ulcer in rats. Furazabol

7) A.D. Bass and J.A. Setliff, *J. Pharmacol. Exptl. Therap.*, **130**, 469 (1960).
8) E.R. Trethewie, *Nature*, **198**, 290 (1963).
9) T. Onodera, M. Ishihara, A. Kasahara and Y. Oshima, *Ohyo Yakuri*, **3**, 191 (1969).
10) T. Onodera, A. Kasahara, M. Ishihara, H. Ogawa and Y. Oshima, *Nippon Yakurigaku Zasshi*, **66**, 458 (1970).
11) T. Akimoto, Personal communication.

had the remarkable curative effect in daily doses of 3 and 9 mg/kg for 10 days. This property may be related to the original anabolic action, but the detail still remains to be investigated.

TABLE IV. Curative Action of Furazabol on Experimental Gastric Stress Ulcer in Rats

Dose (mg/kg/day)	No. of animals	Ulcerated area (mm^2)	Cure of ulcer(%)[a]
Control A (No administration)	10	11.1±2.5[b]	—
Control B (Solvent)	10	12.3±1.7	
3	10	7.4±1.8	39.8
9	10	6.9±1.7[c]	43.9

a) $\frac{\text{ulcerated area (Control B)-ulcerated area (Furazabol)}}{\text{ulcerated area (Control B)}} \times 100$

b) mean ±standard error

c) Significantly different from control B ($p<0.05$, t-test).