LETTER TO THE EDITOR

METALLIC COPPER DECREASES RAT UTERINE PROGESTERONE RECEPTOR CONTENT AND PROGESTERONE BINDING

Sir,

(Received on September 25, 1982)

The mechanism by which metallic copper increases efficacy of an intrauterine contraceptive device still remains unclear. Copper has strong affinity for progesterone receptors and consequently inhibits progestational proliferation (5). However, the change was observed only in the cytosol fraction of the rabbit uterus and no estimate of the effect of copper on nuclear receptors and the translocation process was available. We have therefore investigated these events in the rat uterus.

Healthy female albino rats (150–175 g) were divided into 3 groups of 12 animals each. In one group of animals (C), a pure (95%) copper wire (diameter, 0.2 mm; area, 12.6 mm^2) and in other group of animals (B), a nylon thread of the same specification were fitted in the uterine lumen bilaterally under asceptic conditions. The third group (A) was of sham–operated animals and served as control. The animals were killed by cervical dislocation on 12th day after operation and the uterine horns dissected out. Assay for progesterone receptors and the Scatchard analysis for determining Kd values in the nuclear and cytosol fraction obtained from uterine homogenates, were carried out according to the method standardized previously (3, 4).

The effect of metallic copper on the distribution of radioactive progesterone between cytosol and nuclear fraction was studied *in vitro* using both horns of uteri obtained from additional group of rats. The uteri were incubated with metallic copper wire (see above, for specifications) in 2.0 *ml* of Eagle medium containing 0.05 mmol of ^{3}H-Progesterone at 37°C for various times as indicated (Table II).

At the end of each incubation, the mixtures were cooled and uteri washed twice for 5 min by tris–EDTA buffer containing 0.02 mmol unlabelled progesterone. The uteri were homogenized and centrifuged to get nuclear and cytosol fractions as described

previously (3, 4). The receptor bound activity in the nuclear and cytosol fractions was determined using Packard liquid scintillation spectrometer (Model 33). Protein was determined by the method of Lowry *el al.* (1). Student's *t*–test was employed to assess the significance of difference between groups.

TABLE I : Effect of copper on the progesterone receptor concentration.

Group	*Treatment*		*Nuclei*	*Kdx 10⁻⁹*	*Cytosol*	*Kdx 10⁻⁹*
A	(NIL) Estrus	(6)	156.31± 4.21	2.05±.15	886.33± 91.7	1.09±.03
	Diestrus	(6)	243.21±25.14[a]	2.15± 25	1106.6 ±142.8[a]	1.28±.1
B	Nylon thread	(12)	167.43±5.62	2.22±.10	853.01±78.65	1.5±.21
C	Copper wire	(21)	53.87±8.79	4.50±.20	189.58±22.5[b]	3.55±.17

Values under columns Nuclei and Cytosol are means (±S.E.M.) of bound ^{3}H–progesterone (f *moles/mg* of protein). Number of animals is shown in parentheses.

(a) Significantly different from estrus animal-group ($P<0.01$, t–test)

(b) Significantly different from group A and B ($P<0.001$, t–test).

TABLE II : *in vitro* study of distribution of receptor bound progesterone in control and copper treated uteri.

Incubation Time(min)	*Control uteri*		*Cu–treated uteri*	
	Nuclei	*Cytosol*	*Nuclei*	*Cytosol*
15	1000*	5500	2000	3000
30	2050	6250	2090	3500
45	3500	6350	2075	3558
60	6800	6450	1985	3550

Values represent DPM/uteri (both horns). Incubations were done in duplicate and a total of 5 replicates were used.

The progesterone receptor concentration was found to be higher in the diestrous phase as compared with estrous phase in control rats. This change was uniform for both the fractions, nuclei as well as cytosol (Table I). These findings were in agreement with the earlier reports for rat (6) and guinea pig uterus (2). Progesterone receptor concentration decreased both in nuclear and cytosol fractions in Group C animals. On

the other hand a nylon thread did not show any effect suggesting a qualitative difference between the action of an inert (nylon) and an active (copper) device.

Data presented in Table I revealed that the affinity of the receptor for progesterone was signifiantly altered after copper treatment. An observed increase in the value of Kd in the Group C as compared with Group A and B would suggest a decrease in the binding affinity. This was true for both nuclear and cytosol receptors. This event is likely to affect the process of translocation of the receptor protein complex from cytosol to nucleus. The *in vitro* studies (Table II) also showed that in the control group, there was no noticeable change in the concentration of bound steroid in cytosol fraction after an initial rise at 15 min whereas bound steroid in the nuclear fraction showed an appreciable rise.

On the other hand in copper–treated uteri concentration of the bound steroid in the cytosol and in the nucleus was practically constant during incubations; however, at all times the values for cytosol were higher than in the nuclear fraction. These results would indicate that nuclear binding of the steroid is possibly inhibited by metallic copper which would decrease the translocation process. Copper thus might have a localised effect which results into reduced incorporation of the hormone in the traget organ, thus imparting contraceptive efficacy to a Cu–IUCD.

R. K. JOHRI* AND R. K. PURI**

Division of Endocrinology,
Central Drug Research Institute, Lucknow – 226 001

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* Regional Research Laboratory, Jammu–Tawi - 180 001
** Mayo Clinic, Rochester, Minnessota, U.S.A.