

Effect of Synthetic Double-Stranded RNA on Serum Metals in the Rat, Rabbit, and Monkey¹ (35316)

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(Introduced by R. W. Wannemacher, Jr.)

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Synthetic double-stranded ribonucleic acids, especially the homopolymeric duplex of polyriboinosinic and polyribocytidylic acids (poly I:C), have been shown to share certain similar toxic properties with endotoxins *in vivo*. Both endotoxins and poly I:C are pyrogenic (1), induce the activation and release of preformed interferon in rodents (2), and produce cross tolerance and hyporeactivity for continued interferon production (3). Because endotoxin administration is followed by significant dose-dependent depressions in serum iron and zinc concentrations in various animal models (4-6), it was of interest to determine if Poly I:C also led to changes in trace metal concentrations. The present study reports and compares the effect of poly I:C on serum iron and zinc concentrations in three different animal species.

Materials and Methods. Crystalline polyinosinic acid (poly I), polycytidylic acid (poly C), and homopolymeric duplex of poly I:C were obtained from Miles Laboratories, Elkhart, Indiana, and lipopolysaccharide from *Escherichia coli* 0.127:B8 was obtained from Difco Laboratories, Detroit, Michigan. Male Dunning-Fischer rats weighing 150-175 g, male New Zealand white rabbits, weighing 2.3 or 3.1 kg, and male rhesus monkeys, weighing 2.9 to 4.1 kg were used in this study.

Prescribed doses of the above test compounds were reconstituted in pyrogen-free normal saline. The routes of inoculation were ip in the rat and iv in the rabbit and mon-

key with one exception. One monkey received a dose of 1 mg/kg of poly I:C ip as indicated in Table II.

Blood samples were obtained at 3-hr intervals over a 24-hr period postadministration of the test substances in order to determine time of maximal effect and to correct for any variability associated with diurnal periodicity. Owing to individual variability in base-line values in the rabbit and monkey models, each animal tested served as its own control. Zinc and iron-free polyethylene pipettes, syringes, and test tubes were used throughout the study to minimize contamination of the samples from exogenous sources.

Serum zinc concentrations were determined on a Perkin-Elmer atomic absorption spectrophotometer (Model 303) by a method previously described (6). The colorimetric method of Levy and Vitacca (7) was used to determine serum iron concentrations.

In addition, rectal temperatures were recorded electronically in the rabbit by a telethermometer (Yellow Springs Instruments Co., Yellow Springs, Ohio). Core temperatures were recorded in the monkey by a Honeywell Model 15 recorder via a copper-constantan thermocouple surgically implanted in the para-spinal lumbar musculature so that the sensing tip rested just beneath the posterior abdominal peritoneum.

Results. Table I summarizes the effects of endotoxin, poly I:C, poly I, and poly C on serum zinc and iron concentrations in the rat. As previously reported (4, 6), 100 μ g of endotoxin administered ip produced significant decreases in both serum zinc and iron concentrations with maximal depressions occurring approximately 9 hr post administration. Like endotoxin, 100 μ g of poly I:C also produced significant decreases in the serum

¹ In conducting the research reported herein the investigators adhered to "Guide for Laboratory Animal Facilities and Care" established by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

TABLE I. Effect of Poly I:C, Poly I, Poly C, and Endotoxin on Serum Zinc and Iron Concentrations in the Rat.

Treatment (μg) ^a	Serum zinc ($\mu\text{g}/100\text{ ml}$) ^b	Time of max response ^c	Serum iron ($\mu\text{g}/100\text{ ml}$) ^b	Time of max response ^c
NaCl control	145 \pm 3	—	155 \pm 7	—
Endotoxin, 100	30 \pm 2 ^d	9	39 \pm 7 ^d	9
Poly I:C, 100	81 \pm 4 ^d	6	88 \pm 8 ^d	6
500	66 \pm 3 ^d	9	72 \pm 6 ^d	9
Poly I, 100	147 \pm 2	—	151 \pm 6	—
500	133 \pm 5	—	143 \pm 7	—
Poly C, 100	136 \pm 4	—	149 \pm 7	—
500	141 \pm 6	—	162 \pm 8	—

^a All test compounds were administered ip in 1 ml of pyrogen-free saline.

^b Each value represents the mean of a minimum of 10 animals \pm one SE.

^c Hours postadministration.

^d Values significantly different from control, $p < .01$.

zinc and iron concentrations. At this dose, neither the magnitude of the depressions produced by the poly I:C nor the duration of the effect was as great as that seen with a comparable amount of endotoxin. However, increasing the amount of poly I:C to 500 μg did enhance the magnitude of the depressions. As well as the duration of its effectiveness. By contrast, neither of the two single-stranded polyribonucleotides, at either dose, had any effect on the serum concentration of the two metals.

As shown in Table II, similar results were observed in the rabbit. When 10 $\mu\text{g}/\text{kg}$ of endotoxin was administered iv, serum zinc and iron concentrations were significantly decreased to 29 and 38% of their respective control values, with maximal reductions occurring at 9 hr postadministration. A typical fever response to endotoxin was observed, peaking at 3 hr. Similarly, 100 $\mu\text{g}/\text{kg}$ of poly I:C given iv also produced significant reductions in serum zinc and iron concentrations. Maximal reductions to 53 and 48% of control values were observed at 6 hr postadministration for zinc and iron, respectively. Accordingly, poly I:C, like endotoxin, was shown to be pyrogenic in the rabbit, an observation which confirms the report of Lindsay *et al.* (1). As in the rat model, neither poly I or poly C alone elicited any effect on serum zinc or iron concentrations, nor were they pyrogenic.

In contrast to the rat and rabbit, 500 $\mu\text{g}/\text{kg}$ of poly I:C given iv to the monkey produced neither a change in serum zinc or iron concentrations nor a fever; whereas endotoxin still produced significant serum zinc and iron depressions, as well as a fever (Table II). When the dose of poly I:C was increased to 1 mg/kg and given ip, still no change in serum zinc or iron concentration was observed. However, the latter dose was pyrogenic, with a maximum febrile response occurring in 4 hr postadministration. Again, Poly I and Poly C alone had no effect.

Discussion. The synthetic double-stranded RNA poly I:C has been shown in these studies to share still another endotoxin-like property. Like endotoxin, poly I:C produced significant decreases in serum zinc and iron concentrations in the rat and rabbit with the magnitude and duration of these decreases being dependent upon the dose, as demonstrated in the rat. The mechanism or mechanisms involved in the alterations of zinc and iron metabolism during endotoxemia, acute and chronic infection, and other pathological conditions are still not fully known, but recent evidence suggests that such alterations may be mediated through a common mechanism of host response involving the well-documented changes in reticuloendothelial system activity (8-10). Thus poly I:C may elicit its effect on zinc and iron metabolism in the rat and rabbit in a man-

TABLE II. The Effects of Poly I:C, Poly I, Poly C, and Endotoxin on Serum Zinc and Iron Concentrations and Temperature in the Rabbit and Monkey.

Animal	Treatment ^a	No. of animals	Serum zinc			Serum iron			Febrile response		
			Pre-exposure ^b	Post-exposure ^c	Time of max response ^d	Pre-exposure ^b	Post-exposure ^c	Time of max response ^d	Δ Temp (°)	Time of max response ^d	
Rabbit	NaCl control	3	100 ± 3	98 ± 1	—	100 ± 3	100 ± 4	—	None	—	
	Endotoxin, 10	4	100 ± 4	29 ± 10*	9	100 ± 5	38 ± 11*	9	+2.5	3	
	Poly I:C, 100	3	100 ± 4	53 ± 2*	6	100 ± 2	48 ± 6*	6	+2.4	3	
	Poly I, 100	3	100 ± 3	98 ± 2	—	100 ± 3	100 ± 1	—	None	—	
	Poly C, 100	3	100 ± 3	98 ± 3	—	100 ± 3	100 ± 4	—	None	—	
Monkey	NaCl control	2	100 ± 4	98 ± 2	—	100 ± 5	101 ± 5	—	None	—	
	Endotoxin, 250	2	100 ± 4	68 ± 3*	9	100 ± 6	55 ± 8*	9	+1.5	4	
	Poly I:C, 500	2	100 ± 5	104 ± 2	—	100 ± 6	92 ± 3	—	None	—	
	Poly I, 500	2	100 ± 1	101 ± 5	—	100 ± 5	107 ± 10	—	None	—	
	Poly C, 500	2	100 ± 4	106 ± 3	—	100 ± 8	105 ± 5	—	None	—	
	Poly I:C, 1 (ip)	1	100 ± 4	96	—	100 ± 7	103	—	+1.2	4	

^a All compounds tested were administered iv in 1 ml of pyrogen-free saline; amounts shown (μg/kg), except the last (mg/kg).

^b Preexposure values are expressed as 100% ± 1 SE, and include a minimum of 3 determinations/animal.

^c Postexposure values are expressed as percentage of control ± 1 SE.

^d Hours postadministration.

^e Values significantly different from controls, $p < .01$.

ner similar to that of endotoxin by activating the host defense mechanisms, as made evident by their mutual ability to induce the release of interferon.

However, the question still arises that the poly I:C, in itself, may be contaminated with endotoxin. Although this is a possibility, it seems unlikely, since the single-stranded poly I or poly C elicit no effect. Furthermore, when samples from the same lots of the noneffective single-stranded RNA were allowed to polymerize into the double-stranded poly I:C, under sterile conditions and with the use of pyrogen-free equipment the double-stranded form was typically effective despite being formed from precursors known to be free of endotoxin-like effect.

Although poly I:C has been shown to share another endotoxin-like property, it appears to differ in species specificity, as shown by its noneffectiveness in altering serum zinc and iron concentrations in the rhesus monkey, while still being able to produce fever at the 1 mg/kg dose. Similar results were found by Leonard *et al.* (11), who reported that doses of poly I:C as high as 5 mg/kg in either the patus or rhesus monkey failed to induce interferon release. Such investigations in primates imply that further studies on species-related pharmacologic effects of poly I:C are necessary before this compound can be considered an effective inducer of interferon in man.

Summary. The effect of the synthetic homopolymeric duplex of polyriboinosinic and

polyribocytidylic acids on serum zinc and iron concentration in the rat, rabbit, and monkey was studied. Poly I:C, like endotoxin, was shown to produce significant decreases in serum zinc and iron concentrations when administered to the rat and rabbit. Although poly I:C was found to be pyrogenic in the rabbit and monkey, a species difference was noted by the inability of poly I:C to alter serum zinc and iron concentrations in the monkey.

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