Antagonistic Effect of Ellagic Acid on Histamine Liberators (34101)

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Compound 48/80, dextran, and polymyxin B sulfate are very potent histamine releasers in the rat (1, 2). The dextran reaction differs considerably from the effects of 48/80. The 2-deoxyglucose blocks the anaphylactoid reaction to dextran, but not to 48/80 (3). Similarly diazo-9, 9(dimethylamino-3'-methyl-2'propyl)-10 phenothiazine HCl prevents the blood pressure fall produced by dextran, but only partially inhibited the inflammatory response to 48/80 injection (4). Polymyxin B sulfate releases histamine similar to 48/80, but appears to be more powerful in the rat than 48/80 (2). Papacostas *et al.* (5) studied several compounds in an effort to block the release of histamine, but did not succeed in preventing the lethal effect of 48/80. In this study evidence is shown that ellagic acid blocks the effects of 48/80, dextran, and polymyxin B sulfate *in vivo*.

Materials and Methods. Sixty-six Wistar male rats (200–300 g) from Harlan Indus-

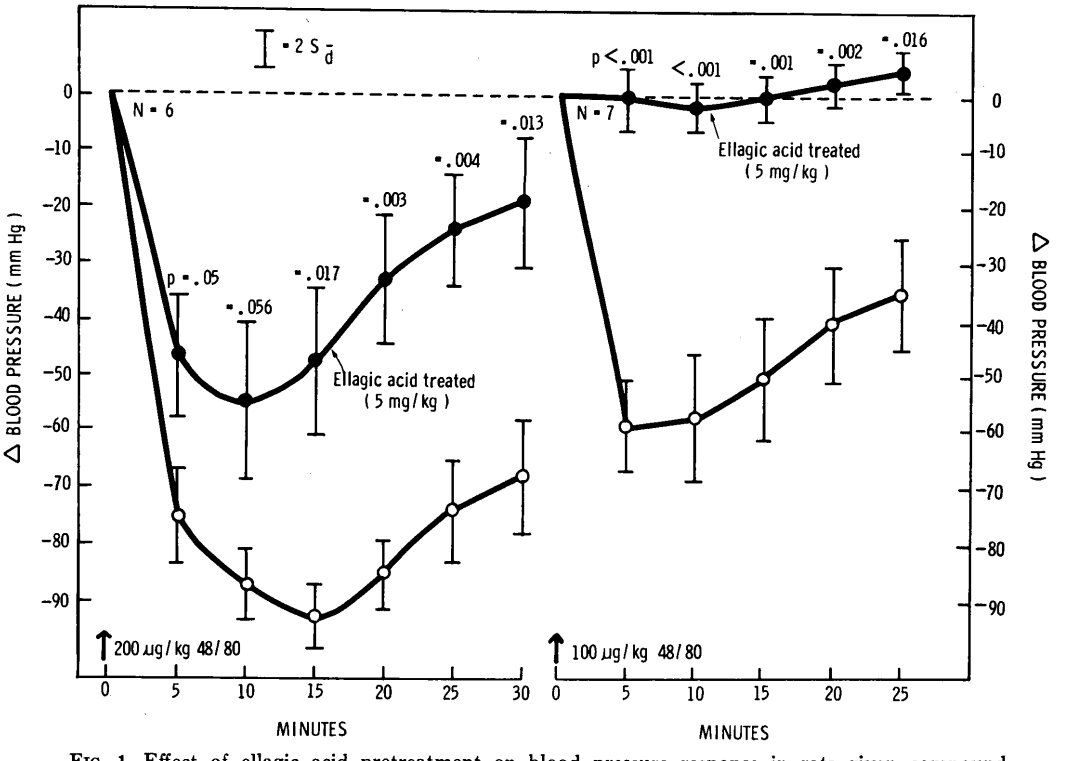

FIG. 1. Effect of ellagic acid pretreatment on blood pressure response in rats given compound 48/80 (200 or 100 µg/kg, iv). Each point represents the mean of six or seven animals ± SE. Statistical significance (p value) was calculated by comparing the mean of the controls with the mean of ellagic acid-treated groups at respective intervals.

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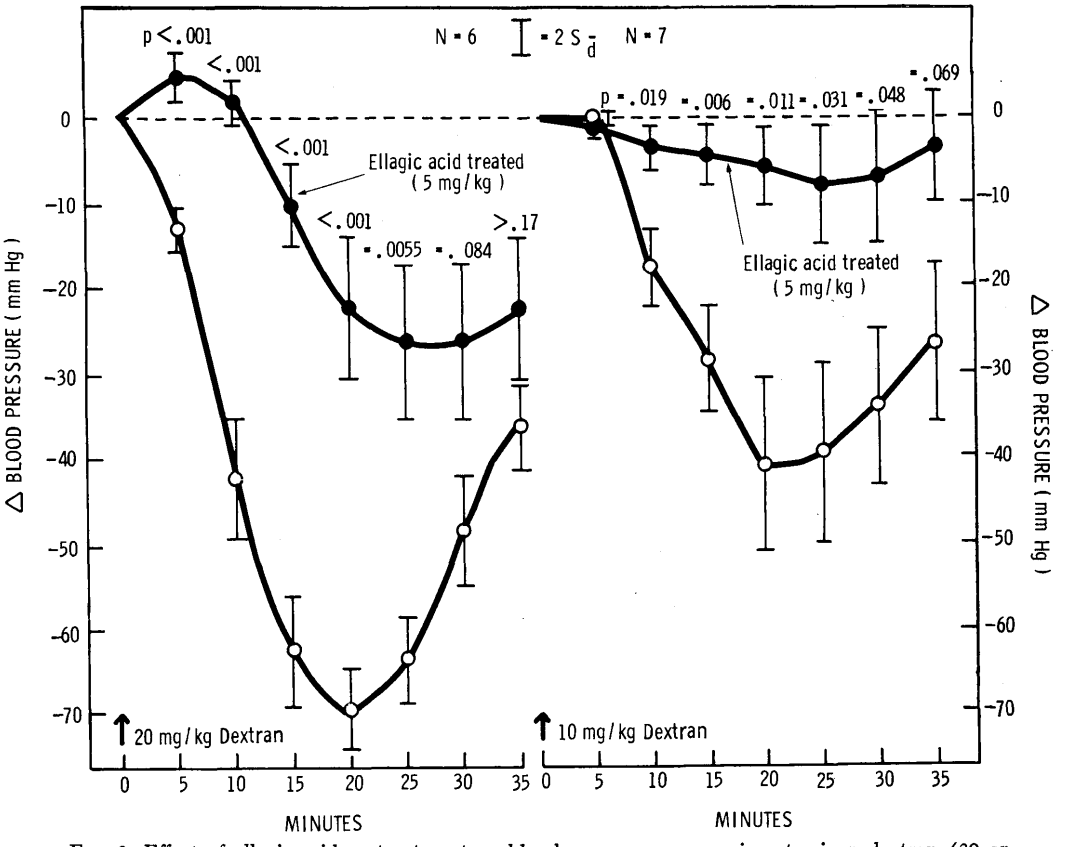

FIG. 2. Effect of ellagic acid pretreatment on blood pressure response in rats given dextran (20 or 10 mg/kg, iv). Each point represents the mean of six or seven animals ± SE. Statistical significance (p value) was calculated by comparing the mean of controls with the mean of ellagic acid-treated groups at respective intervals.

tries and 106 Swiss Webster male mice (22–30 g) from Sutter were maintained in a standard environment and were fed Purina Laboratory Chow and water *ad libitum*.

Ellagic acid was obtained from black walnut (6), dissolved in 0.1 *N* NaOH solution and the pH adjusted to 10 ± 0.5 by HCl. Fresh solutions were prepared each day and kept frozen to reduce decomposition during that day of experimentation.

Rats were anesthetized with 50 mg/kg pentobarbital sodium (ip). Blood pressure was measured through the left carotid artery using a Statham pressure transducer attached to a polygraph recorder (Grass). Drug solutions were injected through the left femoral vein. Rats were treated with ellagic acid (5 mg/kg, iv) approximately 7 min before 48/80, dextran, or polymyxin B sulfate administration. The hematocrit was determined before and 40 min after compound 48/80, dextran, or polymyxin B sulfate administration. In case of the dextran-treated rat, edema was observed in both fore and hind paws.

Results. Figures 1–3 show ellagic acid significantly blocked the blood pressure depression produced by any of the histamine releasers used. Similar results were obtained when twice the amount of 48/80 or dextran was injected. There was a significant antagonism to the hematocrit increase in animals pretreated with ellagic acid, and then given one of the histamine liberators, as compared to controls in which only the histamine releaser was injected (Table I). Only three of seven animals treated with ellagic acid plus dextran showed edema as compared to seven of seven animals that received 10 mg/kg of dextran

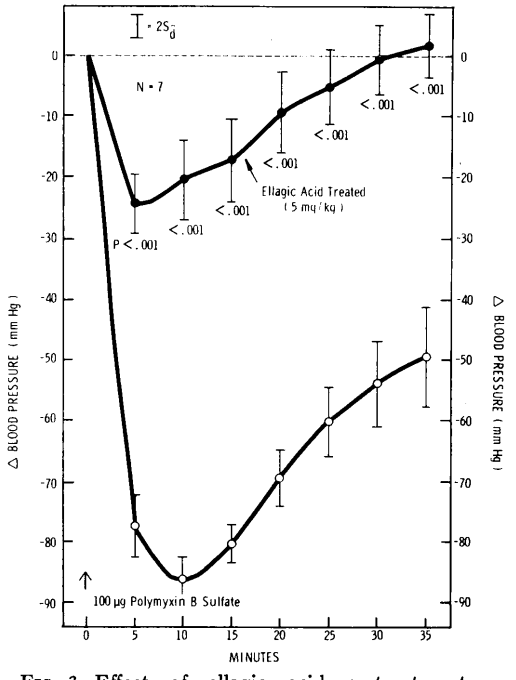

FIG. 3. Effect of ellagic acid pretreatment on blood pressure response in rats given polymyxin B sulfate (100 µg/kg, iv). Each point represents the mean of seven animals ± SE. Statistical significance (p value) was calculated by comparing the mean of controls with the mean of ellagic acid-treated groups at respective intervals.

alone. When 10 µg/kg of histamine phosphate was injected before and after ellagic acid treatment (5 mg/kg, iv), the response to histamine on blood pressure was not significantly changed. Table II illustrates that ellagic acid also antagonizes the lethal effects of compound 48/80 in mice.

Discussion. Results obtained indicate that ellagic acid antagonizes the effect of histamine liberators *in vivo*. This effect is possibly due to ellagic acid interfering with the histamine release mechanism. This conclusion is based upon the fact when histamine was injected after administration of ellagic acid the response to histamine was unaltered. However, when one of the histamine-releasing agents; *e.g.*, 48/80, dextran, and polymyxin B sulfate was injected after the ellagic acid treatment, the fall in blood pressure was significantly reduced. It is already established that the blood pressure fall due to a histamine liberator is the result of histamine release (2, 7). This phenomenon was also supported by other experiments in which ellagic acid antagonizes the hematocrit increase by 48/80, dextran, or polymyxin B sulfate. Such an increase in hematocrit is due to histamine which increases the membrane permeability and thus causes the plasma volume to decrease (8). The data presented in this report also suggest some protection by ellagic acid in mice which may be due to direct antagonism of 48/80, as various workers (5) have suggested that the lethal effect of 48/80 in mice is also related to some toxic action of 48/80 other than histamine release alone.

Prevention of histamine release makes this

TABLE I. Effect of Ellagic Acid on Hematocrit Increase by Histamine Releasers.

Treatment	No. of animals	Per cent hematocrit ± SEM: Control, Before treatment	Control, After treatment	Ellagic acid-treated, Before treatment	Ellagic acid-treated, After treatment	p value[a]
Compound 48/80 (200 µg/kg)	6	42.8 ± 1.19	58.8 ± .98	42.8 ± .48	53.3 ± 1.22	.006
Dextran (10 mg/kg)	7	41.1 ± .96	49 ± 1.7	42.7 ± .95	48.1 ± 1.35	NS
(20 mg/kg)	6	42.8 ± 1.27	55.3 ± 1.69	42 ± .58	50.5 ± 1.57	.06
Polymyxin (100 µg/kg)	7	41.6 ± 1.02	57.6 ± 1.04	43.4 ±1.16	49.8 ± 1.04	<.001

[a] Statistical significance (p value) was calculated by comparing the means of ellagic acid and histamine releaser-treated group with control.

TABLE II. Effect of Ellagic Acid on the Lethal Effect of Compound 48/80 (3 mg/kg iv) in Mice.

Ellagic acid (mg/kg iv)	No. of mice	Mean no. seconds lived ± SEM	Protection (%)
20	10	—	100
15	10	1740 (1 mouse)[a]	90
10	20	1020 (1st mouse)[a] 3600 (2nd mouse)[a]	90
5	10	660 (1 mouse)[a]	90
3.75	10	165 ± 38 (4)	60
2.5	10	255 ± 95 (6)	40
1.25	11	159 ± 42 (10)	9
0	25	106 ± 14 (25)	0

$PD_{50} = 3$ mg/kg

[a] No mean value; number of animals in parentheses.

compound most interesting for a potential therapeutic use. It has also potential in helping us to understand the mechanism of other drugs which reduce blood pressure by releasing histamine.

Summary. Ellagic acid blocks the blood pressure fall induced by potent histamine liberators; namely, compound 48/80, dextran, and polymyxin B sulfate. The extent of edema produced by histamine liberators was also reduced by previous administration of ellagic acid. It also decreased the lethal effect of 48/80 in mice.

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