SHORT REPORT

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TOXICITY OF CYTOCHALASIN E IN FEMALE RATS

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Summary

Intraperitoneal administration of cytochalasin E causes a dose-dependent relationship on the extravascular effusion of plasma into peritoneal cavity of female rats. Lethal dose of cytochalasin E (3.50 mg/kg) had a pronounced effect on a sharp decrease in plasma volume to 38.0 % and the simultaneous accumulation of about 3.00 ml of peritoneal fluid within 1 h after treatment. The cause of death appears to be due to a plasma hypovolumic shock.

Cytochalasin E is a secondary toxic metabolite produced by *Rosellinia necatrix*[1] and *Aspergillus clavatus*[2]. The strain of *A. clavatus* was isolated from leftover cooked rice eaten by a Thai boy who subsequently died with Reye's syndrome[3,4]. The structure of cytochalasin E was proposed by Aldridge *et al.*[1] and corrected structure was proposed by Büchi *et al.*[5] and Aldridge *et al.*[6]. Cytochalasin E was acutely toxic to several animal species including rat, mouse and guinea pig[7]. The i.p. LD_{50} values of cytochalasin E were very similar (2.60 mg/kg) in both male and female adolescent rats. Histopathologic changes revealed degenerative necrosis of liver, kidney, spleen and brain edema. The possible cause of death is a shock resulted from extravascular effusion of plasma into the peritoneal cavity[8]. However, information on the changes in the plasma volume and constituents in female rats treated with cytochalasin E is still lacking. Therefore, the present communication reports an additional data on the toxic effect of cytochalasin E on the fluid balance in female rats.

Adult female Fischer-derived strain rats (180-250 g) from Animal Production Center, Faculty of Science, Mahidol University, were housed in a group of five rats. Animals were offered rat chow (Gold Coin Ltd., Singapore) and water *ad libitum*, and fasted overnight before each experiment.

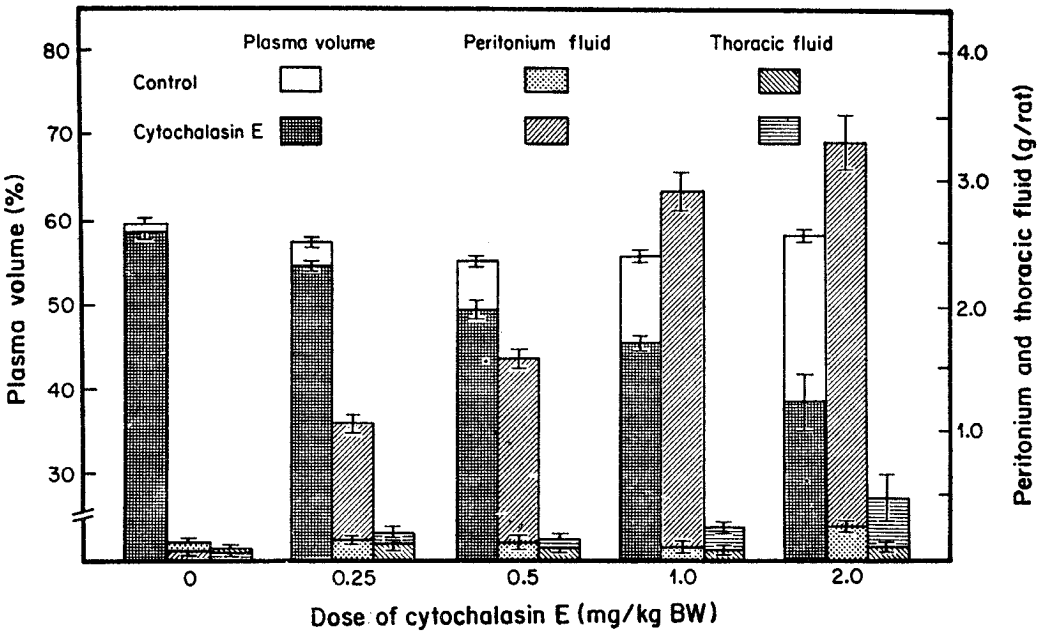

Fig. 1

Plasma volume (percent of whole blood), peritoneal and thoracic fluid changes in adult rats bled at 3 h after treatment with various i.p. doses of cytochalasin E. All results are shown as mean ± S.E.M. Each value is based upon 4 to 7 determinations.

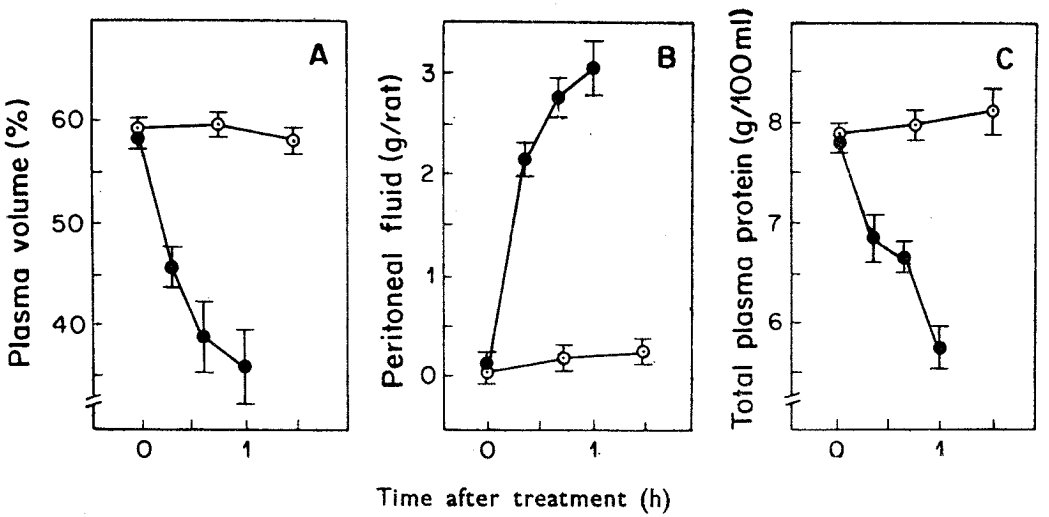

Fig. 2

Plasma volume (percent of whole blood), peritoneal fluid and total plasma protein changes in adult rats bled at various intervals after treatment with lethal i.p. dose of 3.50 mg cytochalasin E/kg. All results are shown as mean ± S.E.M. Each value is based upon 4 determinations. Control, ◉——◉ and cytochalasin E, ●——●)

Cytochalasin E (Sigma Chemical Co., St. Louis, Missouri) was dissolved in dimethylsulfoxide. Peritoneal and thoracic fluids and blood were collected from animals under ether anesthesia. Fluid samples were taken by Hamilton syringe for chemical assay, and the remaining fluid was absorbed into tared cotton pads. Blood was collected from abdominal aorta in oxalate syringe and used for hematocrit determination and preparation of plasma. Total plasma and fluid proteins were determined by method of Lowry *et al.*[9] using bovine serum albumin as the protein standard. Albumin concentrations were determined by the colorimetric method using Sigma albumin color reagent (No. 630-2) with a mixture of human albumin (5% w/v) and globulin (3% w/v) as the protein standard.

TABLE I: TOTAL PROTEIN AND ALBUMIN CONCENTRATIONS OF PLASMA AND PERITONEAL FLUID FROM ADULT FEMALE RATS BLED AT 3 h AFTER TREATMENT WITH VARIOUS I.P. DOSES OF CYTOCHALASIN E.

Compound[a]	Dose (mg/kg)	Plasma		Peritoneal fluid[b]			
		Total protein	Albumin	Total protein		Albumin	
		(g/100 ml)	(g/100 ml)	(mg/g)	Total (mg)	(mg/g)	Total (mg)
DMSO	0.50	7.89 ± 0.09	3.56 ± 0.12	–	–	–	–
Cyto. E	0.25	7.94 ± 0.39	3.67 ± 0.12	56.18±3.96	60.56±0.21	32.95±1.27	34.92±4.22
DMSO	0.50	8.19 ± 0.06	3.94 ± 0.12	–	–	–	–
Cyto. E	0.50	7.53 ± 0.19[e]	3.53 ± 0.13[g]	58.65±0.96	92.57±4.03	31.13±1.88	49.38±4.50
DMSO	0.50	7.74 ± 0.25	3.67 ± 0.11	–	–	–	–
Cyto. E	1.00	6.83 ± 0.14[f]	3.03 ± 0.03[c]	52.20±2.15	153.34±11.18	29.85±1.51	81.67±6.14
DMSO	0.50	7.86 ± 0.34	3.71 ± 0.08	–	–	–	–
Cyto. E	2.00	6.15 ± 0.38[d]	3.13 ± 0.13[d]	55.02±3.14	178.62±4.30	31.06±1.17	101.51±5.38

All results are shown as mean ± S.E.M. Each value is based upon 4 to 7 determinations.

[a] DMSO = dimethylsulfoxide, 0.50 g/kg. Cyto. E. = cytochalasin E.

[b] Insufficient amount of peritoneal fluid precluded analysis in control rats.

[c] $P < 0.001$, [d] $P < 0.005$, [e] $P < 0.01$, [f] $P < 0.025$ and [g] $P < 0.05$.

Toxic effects of cytochalasin E on plasma volume and constituents were studied in groups of four to seven rats treated with i.p. doses of 0.25, 0.50, 1.00 or 2.00 mg/kg. Animals were sacrificed 3 h after treatment. At a dose of 2.00 mg/kg, 2 out of 7 rats died within 2 h after treatment. The dose-dependent loss of plasma was accompanied by a dose-dependent increase in the amount of fluid in the peritoneal cavity, as shown in Fig. 1. The amount of peritoneal fluid was about eleven times

greater than control value after a dose of 0.25 mg/kg, and thirteen times after a dose of 2.00 mg/kg. During this period of treatment, there was approximately six-fold increase in thoracic fluid of the treated rats. The concentrations of total protein and albumin in peritoneal fluid were rather constant at any doses of cytochalasin E (Table I). But the amount of total fluid protein was incresed in accordance with the increase in volumes of peritoneal fluid. Significant loss of total protein and albumin from plasma into peritoneal cavity was closely dose-related (Table I). The similar loss of plasma globulin was also observed, whereas no significant change in plasma fibrinogen was detected (A. Suvannapura and T. Glinsukon, unpublished observation). An 8.1% loss of total plasma protein ($P < 0.01$) was seen at the dose of 0.50 mg/kg and as the dose increased, the loss of total plasma protein was also increased up to 21.7% ($P < 0.005$).

Administration of a lethal dose of cytochalasin E (3.50 mg/kg) caused a sudden increase of the hematocrit up to 64.3% ($P < 0.005$) within 1 h after treatment. According to this change, a rapid fall in the plasma volume was accompanied with an increase in the peritoneal fluid to maximum amount of 3.05 g/rat ($P < 0.005$) (Fig. 2). In addition, significant loss of plasma protein to 5.79 g/100 ml ($P < 0.001$) was also very severe.

The toxicity of cytochalasin E given i.p. to female rats as a powerful plasma hypovolumic agent is well illustrated in this experiment. With respect to dose-dependent relationship for the action of cytochalasin E, these results indicate qualitative similarity to the previous observations using male rats[8]. It is also supported by findings in the present study that two animals treated with 2.00 mg cytochalasin E/kg died shortly after treatment in a similar extent of those study with male rats, and the rapid changes in hematocrit, plasma volume and accumulation of peritoneal fluid after i.p. administration of the lethal dose. Though there are some quantitative differences in lower hematocrit values and higher total plasma and fluid protein concentrations in these female rats which may partly be attributable by sex and strain of rats. However, it suggests that the possible cause of death was a plasma hypovolumic shock resulting from the direct action of cytochalasin E on the venules, capillaries and/or small arterioles[8, 10]. The mechanism of cytochalasin E action on the capillaries is not yet known, however, it is suggested that cytochalasin E like cytochalasin B[11] may dissociate the epithelial cells of capillaries, leading to enlargement of intercellular spaces, and escape of protein and fluid from the circulation. As it has been shown that cytochalasin B dissociates embryonic epithelia of urodeles and chick into a single cell[12].

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บทคัดย่อ

ภายหลังที่ให้ไซโตคาลาซินอีทางช่องท้องของหนูตัวเมียจะทำให้เกิดการไหลซึมของน้ำเลือดเข้าสู่ช่องท้องเพิ่มมากขึ้นตามปริมาณของไซโตคาลาซินอีที่ให้ ทั้งนี้ถ้าให้ปริมาณของไซโตคาลาซินอีขนาดที่ทำให้หนูตายได้ (3.50 มก/กก) จะทำให้เกิดการลดปริมาณของน้ำเลือดในระบบหมุนเวียนโลหิตอย่างรวดเร็วจนเหลือเพียง 38.0 % และในขณะเดียวกันก็จะมีน้ำเหลืองคั่งอยู่ในช่องท้องมากขึ้นถึง 3.0 มิลลิลิตรในเวลา 1 ชั่วโมง ภายหลังที่ให้สารพิษขนาดนี้ สาเหตุของการตายที่ปรากฏนี้คงเนื่องมาจากการช็อคโดยการลดปริมาณของน้ำเลือดอย่างรวดเร็วนั่นเอง