Effects of Nylidrin in Endotoxin Shock

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Abstract □ Endotoxin shock was induced in anesthetized mongrel dogs by the intravenous injection of *Escherichia coli* endotoxin. Although nylidrin, 0.25 mg./kg. administered intravenously at the termination of the initial hypotensive stage, did decrease mortality due to endotoxin-induced shock, the reduction in mortality was not statistically significant. Therefore, it appears that this β-adrenergic stimulant does not prevent the lethal effects of endotoxin-induced shock in dogs.

Keyphrases □ Endotoxin shock—effect of intravenously administered nylidrin □ Nylidrin—effect on endotoxin-induced shock □ β-Adrenergic stimulants—effect of nylidrin on endotoxin-induced shock

Intravenous administration of Gram-negative bacterial endotoxin to dogs produces characteristic circulatory changes including hepatic venoconstriction, portal hypertension, splanchnic pooling, diminished venous return, and lowered arterial blood pressure (1, 2).

Hinshaw and Bradley (3) and Hinshaw *et al.* (4) reported that administration of *Escherichia coli* endotoxin in dogs decreased renal blood flow, glomerular filtration, and tubular transport. Injection of endotoxin directly into the renal artery produced a direct renal vasoconstriction, which could in most instances be prevented by infusion of phentolamine hydrochloride into the renal artery (5). *E. coli* endotoxin may have a direct depressant effect on myocardial contractility (6); however, Maclean and Weil (7) indicated that the initial precipitous fall in blood pressure was due to pooling of blood in the portal system, causing a drop in venous return and a subsequent decrease in cardiac output. More recent evidence suggests that endotoxin does not produce a direct cardiotoxic effect (8).

Mortality in septic shock is high in patients with compromised myocardial or renal function, and deterioration and death are frequently associated with progressive myocardial and renal failure (9). Reversal of these changes has been attempted with a variety of agents, including isoproterenol, a potent β-adrenergic stimulant which reversed the severe hemodynamic and pulmonary alterations in dogs and sheep (10). Starjecki *et al.* (11) reported that isoproterenol reduced mortality of endotoxin shock in dogs and that pretreatment was more effective than treatment after administration of the endotoxin. They found an improvement in cardiovascular function and a marked increase in urine output, suggesting decreased renal resistance.

There are conflicting reports concerning the use of isoproterenol in shock. Isoproterenol may induce myocardial insufficiency because it often produces excessive tachycardia and increased myocardial oxygen consumption. Grega and Kinnard (12) reported that administration of isoproterenol during hemorrhagic shock produced cardiac arrhythmias in some of the dogs, which in certain circumstances leads to ventricular fibrillation.

Nylidrin, another β-adrenergic stimulant, may have some advantages over isoproterenol for it is a less potent cardiac stimulant and produces marked peripheral vasodilatation without causing cardiac arrhythmias or excessive tachycardia. Grega *et al.* (12, 13) reported that nylidrin produced significant protection against hemorrhagic shock in dogs. Nylidrin maintained cardiac output and mesenteric and renal flows during hypovolemic-induced shock while markedly reducing systemic vascular resistance (12, 13). This agent is assumed to produce a more favorable distribution of blood flow by lowering vascular resistance.

The purpose of this study was to investigate the effects of nylidrin in endotoxin-induced shock in dogs.

Table I—Effect of Nylidrin on the Mortality Rate in Endotoxin-Induced Shock in Dogs[a]

Endotoxin Batch	Endotoxin, mg./kg.	Group	Number of Deaths / Number Treated	Death Rate, %
1	2.5	Control	7/10	70
		Treated	2/6	33
2	2.0	Control	5/7	71
		Treated	5/7	71
3	2.0	Control	4/5	80
		Treated	3/5	60
Mean control			16/22	72.7[b]
Mean treated			10/18	55.5[b]

[a] *E. coli* endotoxin. [b] Difference in death rate not significant when data subjected to chi-square test.

EXPERIMENTAL

Adult mongrel dogs, 8–15 kg., of either sex were anesthetized with sodium pentobarbital, 35 mg./kg. i.v., with all experiments conducted on pairs of animals. A femoral artery was catheterized with a polyethylene catheter, and arterial blood pressure was monitored using a transducer[1] connected to a polygraph[2]. All injections were made *via* a femoral venous catheter. A single intravenous injection of 2.0 or 2.5 mg./kg. of body weight of *E. coli* endotoxin[3] in normal saline was given rapidly. One of the pairs of the dogs received 0.25 mg./kg. of nylidrin intravenously when the animal started to recover from the initial episode of hypotension (approximately 30 min. after endotoxin administration), and the other dog received an equal volume of normal saline. Femoral arterial and venous blood samples were collected at selected time intervals; pO_2, pCO_2, and pH were determined using a blood gas analyzer[4]. After 3–4 hr., the incisions in the surviving dogs were sutured closed and the dogs were returned to their home cages and observed for 24 hr. Animals alive 24 hr. following endotoxin treatment were considered survivors.

[1] Statham.
[2] Grass.
[3] Difco.
[4] Radiometer-Copenhagen.

Table II—Percent of Mean Control Systemic Blood Pressure in Dogs[a] Treated with Endotoxin[b]

Group	Control Period, 0 min.	Percent of Mean Control Systemic Blood Pressure ± *SE* after Endotoxin Administration: 5 min.	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.	240 min.
Survived ($N = 7$)	100	46.8 ± 11.6	73.1 ± 6.8	62.4 ± 5.8	49.8 ± 5.5	50.4 ± 6.5	52.5 ± 5.3	58.2 ± 4.8	70.5 ± 5.3
Dead ($N = 12$)	100	56.1 ± 8.0	73.5 ± 4.1	54.8 ± 5.1	40.9 ± 3.1	40.9 ± 2.8	45.7 ± 3.2	46.7 ± 3.3[c]	53.9 ± 4.3[c]

[a] Only dogs surviving more than 240 min. after endotoxin are included. [b] Data include both nylidrin-treated (0.25 mg./kg.) and saline-treated animals. [c] These values are significantly lower than the corresponding values from the group of dogs surviving when data were subjected to Student's t test ($p < 0.05$).

Table III—Percent of Mean Control Systemic Blood Pressure in Control Dogs and in Dogs Treated with Nylidrin (0.25 mg./kg. i.v.) 30 min. after Endotoxin[a]

Group	Control Period, 0 min.	Percent of Mean Control Systemic Blood Pressure ± *SE* after Endotoxin Administration: 5 min.	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.	240 min.
Control ($N = 11$)	100	53.7 ± 8.8	73.4 ± 4.8	53.2 ± 5.1	38.6 ± 2.9	40.1 ± 2.9	46.4 ± 3.5	49.2 ± 3.7	59.9 ± 4.6
Treated ($N = 8$)	100	51.4 ± 10.4	73.3 ± 5.3	63.7 ± 5.6	51.8 ± 4.6[b]	50.3 ± 5.7	50.7 ± 4.8	53.2 ± 4.9	60.1 ± 6.6

[a] Only dogs that survived more than 240 min. after endotoxin are included. [b] This value is significantly higher than the corresponding control value when data were subjected to analysis using Student's t test ($p < 0.025$).

RESULTS

The effects of nylidrin on the mortality of endotoxin-induced shock are summarized in Table I. Preliminary data obtained with the first batch of endotoxin suggested that nylidrin afforded protection against the lethal effects of the toxin; however, subsequent experiments indicated that the compound failed to attenuate the lethal effects of endotoxin-induced shock in dogs. Statistical analysis of the pooled data using the chi-square test indicated that nylidrin did not significantly reduce mortality induced by *E. coli* endotoxin. The percents of the mean control systemic blood pressure of dogs that died and of those that survived the endotoxin shock are shown in Table II. In both groups of dogs, blood pressure fell sharply following administration of the endotoxin and then slowly recovered to approximately 73% of the control value at 30 min. Systemic blood pressure of the dogs that eventually died dropped to 40.9% of the control value 90 min. following administration of the endotoxin and then slowly recovered to 53.9% of control levels at 240 min.

In the dogs that survived the endotoxin shock, blood pressure was not as markedly affected, reaching 49.8% of control values at 90 min. and then slowly recovering to 70.5% of the control level 240 min. following endotoxin administration. The arterial blood pressure in the surviving animals was significantly higher at 180 and 240 min. after endotoxin administration than in those animals that eventually died ($p < 0.05$). Examination of the time course of the mean systemic blood pressure of control and nylidrin-treated animals (Table III) indicated that administration of nylidrin appeared to have slowed the rate of development of the hypotensive stage. The systemic blood pressure in nylidrin-treated animals reached a value of 51.8% of control and was significantly higher than the corresponding value of 38.6% of control blood pressure in the animals that were not treated ($p < 0.025$). However, in the later stages, there was no difference between the two groups of animals.

Arterial acidosis developed in all animals following endotoxin administration, and nylidrin failed to correct this alteration in blood pH. There was a decrease in arterial pCO_2, which was slightly greater in the nylidrin-treated group, suggesting respiratory compensation of the metabolic acidosis.

DISCUSSION

These studies indicate that nylidrin did not significantly reduce mortality caused by *E. coli* endotoxin, although isoproterenol, another β-adrenergic stimulant, was reported to be effective (10, 11, 14). Knowledge of the basic mechanisms involved in the onset and progression of irreversible endotoxin shock is incomplete. Various vasoactive hormones which are released in an attempt to compensate for, or as a result of, the delayed effects of the endotoxin have been implicated (15) in endotoxin shock. A deficient blood supply to vital organs, either as a result of excessive vasoconstriction or of precapillary shunting, was reported as the major cause of irreversible shock (1). Stagnation of blood and increased hematocrit are other contributing factors (2). It was reported by this laboratory (12, 13) that nylidrin is capable of protecting dogs against hemorrhagic shock by maintaining cardiac output and mesenteric and renal flows in the presence of hypotension. It also lowered systemic vascular resistance, prevented GI distension and necrosis, and decreased the degree of metabolic acidosis. The lack of efficacy of this drug in endotoxin shock reflects the complexity of this shock syndrome.

Systemic blood pressure changes following endotoxin administration in these experiments were similar to the changes reported in the literature. The course of blood pressure in dogs that survived endotoxin shock differed somewhat from that of the animals that eventually died. The second phase of hypotension in the surviving animals developed more gradually, and the degree of hypotension was not as profound as in the other group of dogs. This severe and prolonged hypotension appears to be an important factor contributing to the mortality in endotoxin shock. The precipitously low blood pressure would cause a profound decrease in tissue perfusion, thereby causing irreversible damage and eventually death. In those animals that survived, the systemic blood pressure, although lower than control values, was still significantly higher at 180 and 240 min. following endotoxin administration than in the dogs that died within the 24-hr. observation period.

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Structure of Sanguidimerine, a New Major Alkaloid from *Sanguinaria canadensis* (Papaveraceae)

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Abstract □ Structure elucidation of a new major alkaloid, sanguidimerine, was based on the interpretation of its physical and chemical characteristics and was verified by partial degradation and semisynthesis. The possibility of the natural occurrence of sanguidimerine in *Sanguinaria canadensis* is also discussed.

Keyphrases □ *Sanguinaria canadensis* (Papaveraceae)—structure elucidation of sanguidimerine □ Sanguidimerine, alkaloid from *Sanguinaria canadensis*—structure □ Alkaloids—structure of sanguidimerine isolated from *Sanguinaria canadensis*

During a continuing search for anticancer compounds from plants, an investigation was initiated on the rhizomes of *Sanguinaria canadensis* because of its reputed use as a folk remedy and because certain alkaloids (*e.g.*, sanguinarine and chelerythrine) present in *S. canadensis* have been shown to possess antitumor activity (1). The study resulted in the isolation of sanguinarine and a new alkaloid designated as SC-2, which was assigned the trivial name sanguidimerine (2).

This report concerns the structure elucidation of sanguidimerine.

DISCUSSION

Sanguidimerine (I), a crystalline base, m.p. 174°, $[\alpha]_D^{26}$ +21.2° (concentration 0.5 in pyridine), was found to be only slightly soluble in most organic solvents and insoluble in water. It was determined earlier (2) that the UV absorption of this base (Table I) was very similar to that of the benzophenanthridine-type alkaloids and, particularly, to that of dihydrosanguinarine (3). A nonconjugated carbonyl was evidenced by a strong absorption at 1710 cm.$^{-1}$ in the IR spectrum.

Analysis by means of high resolution mass spectrometry (Tables II and III) showed that I exhibited a molecular peak at *m/e* 720, corresponding with a molecular formula of $C_{43}H_{32}N_2O_9$ (Table III). During the fragmentation (Scheme I), hydrogen transfer occurred, producing an ion at *m/e* 389 which was consistent as being identical to acetonyl dihydrosanguinarine, $C_{23}H_{19}NO_5$, and which further fragmented to give a base peak at *m/e* 332 This latter peak was verified by high resolution measurements (Table III) to be $C_{20}H_{14}NO_4$, *i.e.*, the sanguinarium ion (ion *b*). Formation of the sanguinarium ion was further evidenced by the presence of some diagnostic ions, *c*, *d*, and *e*, at *m/e* 317 (ion *b* − 15). 259 (ion *b* − 15 − 58), and 201 (ion *b* − 58 − 58 − 15), which were previously observed in the mass spectrum of sanguinarine pseudocyanide and related bases by Slavik *et al.* (4).

Treatment of I with hydriodic acid yielded sanguinarine as the iodide, and the stoichiometric ratio suggested that one part of sanguidimerine (I) produced two parts of sanguinarine iodide, which further established that the two parts of the dimer were identical (dihydrosanguinarine moiety) and were linked together by means of a C_3H_4O group which contained a carbonyl function.

Previously, a similar benzophenanthridine dimer alkaloid (5) was isolated from *Cheliodonium majus*. Upon comparison, it was found that these two alkaloids, sanguidimerine (I) and chelidimerine, provided identical IR, UV, and mass spectra. However, they differed with respect to melting points (174° *versus* 258–260°), solubility characteristics, and optical rotations. While sanguidimerine was only slightly soluble in most organic solvents, chelidimerine was readily soluble in these solvents. A positive specific rotation was observed for sanguidimerine, whereas chelidimerine was optically inactive. It was, therefore, logical to assume that these two bases were isomers, sanguidimerine being the optically active (+) compound. Preliminary single-crystal X-ray crystallographic data suggested that chelidimerine was a *meso*-compound (5). The data thus suggested sanguidimerine (I) to be (+)-1,3-bis(11-hydrosanguinarinyl)acetone.

Table I—UV Spectra of Sanguidimerine and Dihydrosanguinarine

Alkaloid	λ_{max}, nm. (log ε)	Reference
Sanguidimerine	235 (4.65); 284 (4.63); 323 (4.23)	2
Dihydrosanguinarine	236 (4.62); 284 (4.62); 323 (4.28)	3

Table II—Mass Spectrometric Data for Sanguidimerine

m/e	Abundance, %[a]
720	4
389	2
362	2
332	100
317	12
316	2
274	4
259	3
201	3

[a] Spectra were obtained using a double-focusing mass spectrometer MS9, Allied Electrical Instrument Industries Ltd., Manchester, England.