Effect of Prooxidants on the Survival of Rabbits Exposed to *Staphylococcus aureus* Toxic Shock Exotoxin and on the Chemiluminescence of Their Whole Blood Cells *in Vivo*

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The prooxidant Adriablastin (Adriamycin) is shown to prolong the survival of Chinchilla rabbits exposed to the toxic shock exotoxin produced by *Staphylococcus aureus* as well as to exert a marked effect on the time course of both spontaneous and opsonized zymosan-stimulated chemiluminescence by whole blood cells of these animals.

Key Words: *toxic shock exotoxin; chemiluminescence; prooxidants*

The toxic shock exotoxin (TSE) produced by *Staphylococcus aureus* is now generally recognized as the principal etiologic agent of toxic shock syndrome [4], although the molecular mechanisms involved remain to be elucidated. In particular, the question of how TSE influences the production of reactive oxygen species by phagocytes has not been addressed. In view of this, the objectives of our study were to examine TSE for its influence on the chemiluminescence (CL) of whole blood cells *in vivo* and to test the possibility of utilizing pro- or antioxidant agents for pharmacological correction of the toxic shock induced by this exotoxin.

MATERIALS AND METHODS

The LD_{50} of TSE was determined as described by Lakin [2] in male Chinchilla rabbits (body weight 1000±100 g), and rabbits of this breed were then used throughout the study. A TSE solution was injected into rabbits intraperitoneally, and the number of dead animals was recorded 24 h later. For further work, series of TSE solutions killing 50% of the rabbits were used, in doses not higher than 200 μg of the base substance (in terms of protein [6]) per kilogram of body weight. Test rabbits received Adriablastin [synonym: Adriamycin] (1 mg/kg), catalase (40,000 U/kg), or tocopherol (1 mg/kg) in addition to TSE at the LD_{50} level. Rabbits injected with TSE alone at this level served as controls; CL of their blood cells was compared to that of blood cells from the groups of test and intact rabbits.

The activity of polymorphonuclear leukocytes was evaluated by CL of whole blood because its CL is due almost entirely to this class of cells [7]. CL was determined with a thermostatically controlled (37°C) chemiluminometer (manufactured by the *Dialog* Joint Enterprise, Russia), as previously described [5], in blood samples diluted 1:50 in Hanks' solution without phenol red [3]. Blood samples were taken by puncturing the auricular vein at 45, 180, and 360 min after TSE injection. Deaths in the control and test groups were recorded 24, 48, and 72 h and then 7 days postinjection.

The results were statistically analyzed by conventional methods [2].

RESULTS

The prooxidant Adriablastin [1] considerably prolonged survival times in the test group, whereas the antioxidants catalase and tocopherol did not have an appreciable effect on the survival of test rabbits (Table 1).

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TABLE 1. Effect of Anti– and Prooxidants on the Survival of Rabbits Exposed to Toxic Shock Exotoxin (TSE) from *S. aureus* ($n=8$)

	Time after TSE injection											
	24 h			48 h			72 h			7 days		
Control rabbits												
Survived	4			3			3			2		
Died	4			5			5			6		
Test rabbits												
Subgroup	ADR	CAT	TOC	ADR	CAT	TOC	ADR	CAT	TOC	ADR	CAT	TOC
Survived	8*	4	3	8*	3	3	8*	3	3	7*	3	2
Died	0	4	5	0	5	5	0	5	5	1	5	6

Note. *$p<0.05$ in comparison with the control rabbits. ADR = Adriablastin; CAT = catalase; TOC = tocopherol.

TABLE 2. Effect of Adriablastin on Spontaneous and Luminol–Mediated Chemiluminescence of Whole Blood Cells and on the Chemiluminescent (CL) Response of These Cells to Opsonized Zymosan in Rabbits ($n=8$) Exposed to Toxic Shock Endotoxin (TSE) from *S. aureus*

Time after TSE injection, min	Spontaneous CL, rel. units	Maximal CL response of cells to opsonized zymosan[1]
Control rabbits		
45	5.86±1.81**	5.47±1.12**
180	5.98±1.75**	7.96±1.02**
360	8.47±2.11	1.73±0.82**
Test rabbits		
45	40.12±3.39*	3.08±0.48
180	28.14±5.23*	3.66±0.91*
360	26.73±2.08*	4.76±0.84*
Intact rabbits		
	13.34±2.42	16.65±2.02

Note. [1]As calculated by the formula $[(J-J_0)/J_0]_{max}$, where J_0 is the spontaneous CL and J is the CL after zymosan administration. *$p<0.05$ in comparison with the control rabbits; **$p<0.05$ in comparison with the intact rabbits.

At 45 and 180 min after the injection of TSE, spontaneous CL of blood cells in the control group was significantly lower than in the group of intact animals (Table 2). Similar differences between these two groups were noted at 45, 180, and 360 min postinjection for the CL stimulated by opsonized zymosan. In sharp contrast, spontaneous CL in the subgroup of test rabbits administered Adriablastin was significantly higher than in the control group at all times when it was measured. Although the opsonized zymosan-stimulated CL in this subgroup was always considerably below the control values, there was no tendency toward a marked reduction of its level by minute 360 after toxin injection.

Thus, the prooxidant Adriablastin is capable of reducing mortality among animals exposed to TSE. This effect may be due to the ability of Adriablastin to activate generation of reactive oxygen species by blood phagocytes.

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