PHARMACOLOGY AND TOXICOLOGY

Dermaprotecting Properties of Sodium Succinate under Conditions of Impaired Circulation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 10, pp. 420-424, October, 1998
Original article submitted January 13, 1997

Sodium succinate improves the survival of a skin graft in mice, rats, and dogs, normalizes histamine and serotonin concentrations in the epidermis and blood, exhibits antitoxic activity, improves microcirculation in the skin, brain, heart, kidneys, and testes without any appreciable effect on systemic arterial pressure, cardiac function, and liver blood flow in rats.

Key Words: *sodium succinate; dermaprotecting activity; microcirculation*

Microcirculation disorders accompanying skin plasty and leading to partial or complete necrosis of the recipient's skin or skin graft [1] can be corrected by drugs that increase the viability of ischemized skin and by preventing and correcting microcirculatory disorders.

Sodium succinate (SS) has been used as an endogenous adaptogen that increases tissue resistance to damaging factors [2,6]. It was suggested that it can be employed as an agent preventing inflammation and stimulating the growth of the epithelium [3,5]. By improving oxidation and energy metabolism in various tissues, SS may play an substantial role in detoxifying therapy [2,4,7,8].

In the present study we assessed the dermaprotective activity of SS and examined SS effects on systemic and regional hemodynamics.

MATERIALS AND METHODS

Experiments were performed on 159 mice, 237 rats, and 48 dogs. The acute toxicity doses (LD_{50}) were determined in mice. The antinecrotic activities of SS and of the reference preparations lithium succinate and lithium oxybutyrate (this compound possesses high dermaprotective activity [11]) were assessed in mice, rats, and dogs as described [9]. The antinecrotic activity was regarded as the ability of the studied compounds to improve the survival of a pedicle skin graft. The mean effective dose (ED_{50}) and antinecrotic index (LD_{50}/EF_{50}), which characterize the spectrum of the studied pharmacological effect.

The effect of SS on the contents of the inflammation mediators histamine and serotonin in the epidermis and blood was examined in male albino rats. Twenty-four hours before the experiment, skin on the back was depilated with 10% sodium sulfate. Pedicle skin graft was taken from rats under sodium pentobarbital anesthesia (40 mg/kg intraperitoneally). Experimental animals were injected with SS (5% solution, 100 mg/kg) on the day of the surgery and for two days after it. Control rats were injected with an equal volume of normal saline. The rats were decapitated after 3 days. Parallel measurements were performed in experimental, control, and intact rats. The histamine [12] and serotonin [13] contents in the graft epidermis were measured spectrofluorimetrically at least in 3 rats.

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0007-4888/98/0010-01017$20.00

TABLE 1. Antinecrotic Activity of SS, Lithium Succinate, and Lithium of Oxybutyrate in Mice and Rats

Compound	Animals	Antinecrotic activity			Acute toxicity			Antinecrotic index LD_{50}/ED_{50}
		ED_{50}		relative	ED_{50}		relative	
		mg/kg	mmol/kg		mg/kg	mmol/kg		
SS	Mice (*n*=33)	57.5 (38.5-70.5)	0.355	8.9	6500	40.11	0.3	113.0
	Rats (*n*=39)	51.5 (35.5-69.0)	0.318	13.6				126.2
Lithium succinate	Mice (*n*=30)	167.4 (140.3-196.9)	1.350	2.3	3986	32.14	0.3	23.8
	Rats (*n*=30)	142.7 (120.4-162.6)	1.151	3.8				27.6
Lithium oxybutyrate	Mice (*n*=36)	347.9 (313.7-382.0)	3.16	1	1265[1]	11.49	1	3.6
	Rats (*n*=38)	478.2 (349.1-602.1)	4.34	1				2.6

Note. ED_{50} was determined taking into account the area of survived skin graft. LD_{50} was determined in mice after intraperitoneal injection. The confidence limits at p=0.05 are given in parentheses. The parameters were compared at doses calculated in mmol/kg. The antinecrotic indexes are calculated with LD_{50} for mice. Data of I. V. Aleksanyan *et al.* [1].

Serum activities of alanine (AlAT) and aspartate aminotransferases (AsAT) were measured by the method [14].

Changes in systemic and regional hemodynamics were studied by the method of radiolabeled microspheres in 10 awake rats [10]. Intact rats (n=10) served as the control. The microspheres were labeled with ^{57}Co, ^{113}Sn, or ^{46}Sc and injected 3 times at 24-h intervals. Sodium succinate (5% solution intravenously) or an equal volume of normal saline (control rats) was injected within the 24-h intervals. Heart rate and systemic arterial pressure were recorded with an SR-01 electromanometer, 566 and 567 Hugo Sachs amplifiers, and a Mark VII recorder (Graphtec). After the experiments, the rats were euthanized with sodium pentobarbital overdose. Skin, heart, brain, liver, kidneys, and testis specimens were collected, weighed, and placed in plastic vials. The amount of microspheres was determined in a Compu Gamma-1282 g-counter 1282 (LKB-Wallac). Cardiac index (ml/min/100 g) and flow rate (ml/min/g tissue) were calculated using Super-Calc-2 software.

The results were analyzed by the standard statistical methods using Student's t test for paired samples of independent series.

RESULTS

Antinecrotic activity and the therapeutic spectrum broadness of intraperitoneally administered SS were, respectively, 8.9- and 13.6-fold (mice) and 31.4- and 48.5-fold (rats) greater than those of lithium oxybutyrate (Table 1). Under the chosen experimental conditions, the antinecrotic activity of lithium succinate in mice and rats (3.8- and 3.2-fold, respectively) times) and the broadness of therapeutic spectrum (4.7- and 4.6-fold) were lower than those of SS, and surpassed those of lithium oxybutyrate: 2.3- and 3.8-fold (antinecrotic activity) and 6.6- and 10.5-fold (therapeutic spectrum broadness).

Sodium succinate improved the skin graft survival in dogs (Table 2). After a single intravenous dose of 100 mg/kg the graft survival increased by 33.2% in comparison with the control and by 76.7% after three injections (100 mg/kg intravenously on the first day and 200 mg/kg intramuscularly for 2 days).

Thus, SS improved the survival of a skin graft in mice, rats, and dogs, its antinecrotic activity being higher after three injections than after a single injection.

An inflammatory reaction developed in the skin graft epidermis, as evidenced by increased contents

TABLE 2. Effect of SS on the Survival of Skin Graft in Dogs ($M\pm m$, n=12)

Compound, mg/kg	Administration route	Days of administration	Graft state	
			necrotic area, %	survived area, %
Normal saline	i.v.	1	77.5±3.70 (69.4-85.6)	
SS, 100	i.v.	1	51.8±4.81* (41.4-62.4)	33.2
Normal saline	i.v.+i.m.	1+2 and 3	76.0±5.18 (64.6-87.4)	
SS, 100+200+200	i.v.+i.m.	1+2 and 3	17.7±3.33* (10.4-25.0)	76.7

Note. The confidence limits at p=0.05 are given in parentheses. i.v. intravenously; i.m. intramuscularly. [1]compared with the control. *p<0.001 compared with the control.

TABLE 3. Histamine and serotonin Contents in the Rat Epidermis (*M±m*, *n*=6)

Rats	Epidermis, mg/g		Blood, mg/ml	
	histamine	serotonin	histamine	serotonin
Intact	3.851±0.129	3.504±0.229	0.252±0.009	0.533±0.012
With isolated skin graft	6.133±0.130*	4.372±0.195*	0.193±0.006*	0.346±0.016*
With isolated skin graft+SS	4.102±0.108	4.181±0.236	0.267±0.005	0.528±0.017

Note. *$p<0.05$ compared with intact rats.

TABLE 4. Serum AlAT and As AT Activities in Rats (*M±m*)

Rats	AlAT	AsAT
	μmol/ml/h	
Intact	0.89±0.005 (100)	1.23±0.03 (100)
With isolated skin graft	1.40±0.02 (157.3)*	1.58±0.07 (128.5)*
With isolated skin graft+SS	1.07±0.01 (120.2)**	1.30±0.01 (105.7)***

Note. All groups contained 6 rat, except intact rat (n=7) in the AsAT assay. Percentage is given in parentheses. *$p<0.001$, **$p< 0.01$, ***$p<0.05$ compared with intact rats.

of histamine (59.3%) and serotonin (24.8%) in comparison with those in intact animals. In rats given three injections of SS these parameters practically did not differ from the control (Table 3).

In rats with the skin graft, blood levels of histamine and serotonin decreased 76.5 and 64.9%, respectively, in comparison with intact animals (Table 3). Sodium succinate normalized these parameters to the levels observed in intact animals.

Serum AlAT and AsAT activities are indicators of the liver functional state. In animals with skin grafts the enzyme activities increased 57.3 and 28.5%, respectively, (Table 4), indicating that endotoxins produced in the graft during its necrosis have a negative effect on the liver. This was confirmed by a decrease in the AsAT/AlAT activity ratio from 1.382 (in intact animals) to 1.29. After three injections of 5% SS solution this ratio increased to 1.215.

Dermatoprotecting preparations have been used in the treatment of burns. Since extensive burns lead not only to local skin damage but also to generalized disorders (massive plasma loss causes microcirculatory and functional disorders in the brain, heart, liver, kidneys etc.), it was reasonable to examine the effects of SS on microcirculation and hemodynamics in various organs.

After two intravenous injections of SS (100 mg/kg), skin blood flow increased and vascular resistance in the skin, left hemisphere, heart, both kidneys, and both testes decreased (Figs. 1-3). There were no statistically significant changes in systemic arterial pressure, heart rate, cardiac output, stroke volume, total peripheral resistance, and liver blood flow and vascular resistance. Under the effect of SS some parameters of cardiac activity increased: cardiac output by 26.5%, cardiac index by 23.1%, and stoke

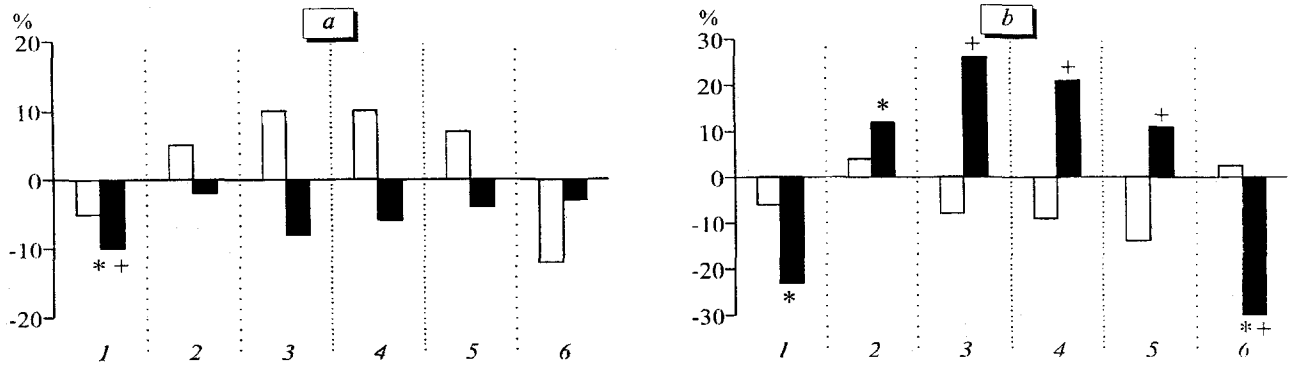

Fig. 1. Effect of sodium succinate (100 mg/kg intravenously, two times at a 24-h interval) on systemic and regional hemodynamics in control (*a*) and experimental (*b*) rats. *1)* systemic arterial pressure; *2)* heart rate; *3)* cardiac output; *4)* cardiac index; *5)* stroke volume; *6)* total peripheral resistance. Here and in Figs. 2 and 3: white bars: 24 h after the first injection of substance; black bars: 24 h after the second injection. $p<0.05$: *compared with the initial value; +compared with the value after the injection of substance.

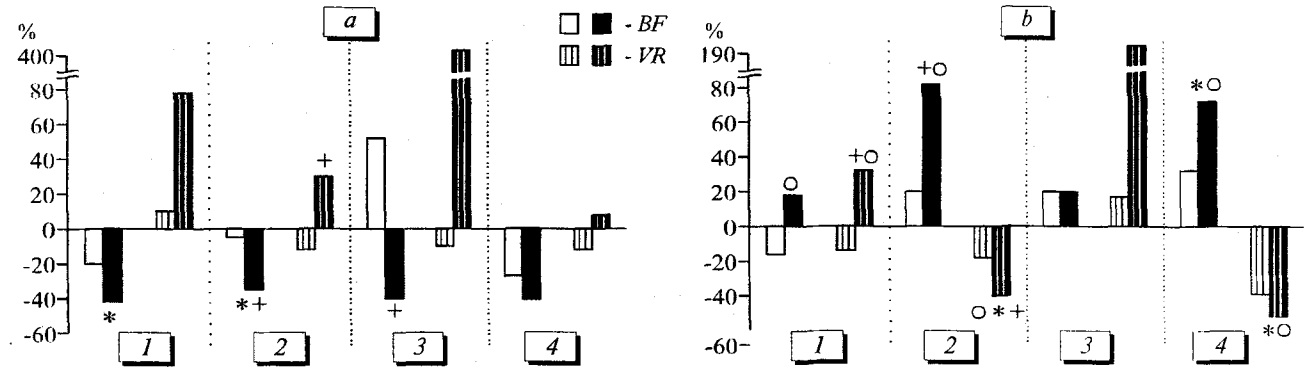

Fig. 2. Effect of sodium succinate (100 mg/kg intravenously two times at a 24-h h interval) on blood flow (*BF*) and vascular resistance (*VR*) in the skin *(1)*, left brain hemisphere *(2)*, liver *(3)*, and heart *(4)* in control *(a)* and experimental *(b)* rats. Here and in Fig. 3: °$p<0.05$ compared with the corresponding values in the control.

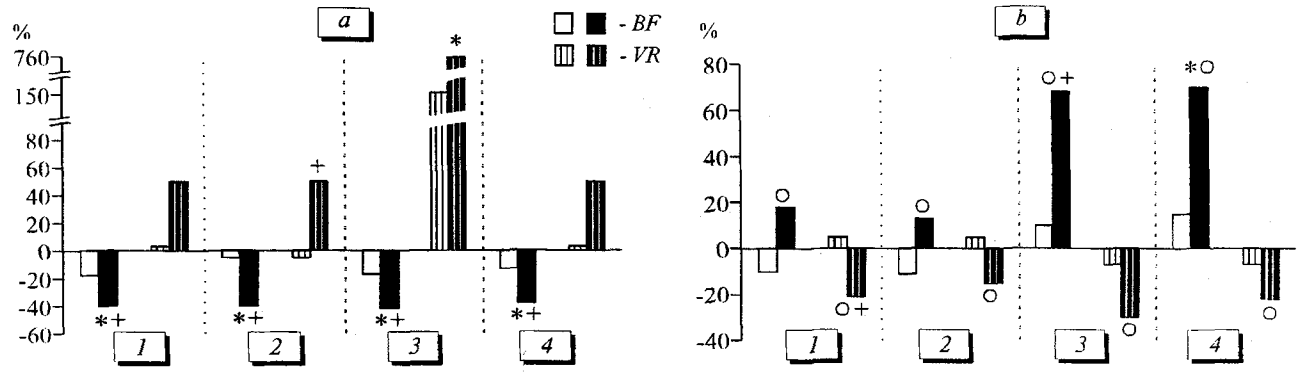

Fig. 3. Effect of sodium succinate (100 mg/kg intravenously two times at a 24-h h interval) on blood flow (*BF*) and vascular resistance (*VR*) in the left *(1)* and right *(2)* kidney and left *(3)* and right *(4)* testis in control *(a)* and experimental (*b*) rats.

volume by 17.7%. Sodium succinate improved myocardial microcirculation: blood flow rate increased by 75.6% and vascular resistance decreased by 47.6%). The liver blood flow rate increased by 118%, while vascular resistance decreased by 75.2%.

Thus, our findings indicate that SS improves the survival of a skin graft in various animal species, normalizes the contents of histamine and serotonin in the epidermis and blood, exhibits antitoxic activity, improves microcirculation in the skin, brain, heart, kidneys, and testes without no appreciable effect on systemic arterial pressure, cardiac function, and liver blood flow.

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