POLAROGRAPHIC STUDIES OF BLOOD SERUM OF ANIMALS TREATED WITH SILICIC ACID

Modi, Iene, Sabo, Ishtvan, and N. Chiki Ilona

Department of Normal Physiology (Head, Doctor Sabo Ishtvan)
and Department of Biochemistry (Head, Doctor Kovach Éndre),
Tyrgu-Mureshskii Medical Institute, Roumania
Presented by Active Member AMN SSSR V. V. Parin
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The destructive action of colloidal silicic acid on tissues plays an important part in the pathogenesis of silicosis. This acid forms in the tissue fluids when silicon dioxide penetrates into them. Its influence on the organism is shown chiefly in the denaturation of proteins, as has been demonstrated in vitro. The pathological changes consist in the formation of silicotic nodules. There is a dystproteinemia, which indicates disturbances of protein metabolism. This effect was confirmed by polarographic studies of blood serum and its proteins [1, 8, 13]. However, until now the mechanism of these disturbances has remained unknown. In particular, it is not known whether they are due only to the presence of silicic acid.

The present study is intended to show what changes occur in the polarogram of the serum of animals treated with silicic acid or with amorphous silicon dioxide.

EXPERIMENTAL METHOD

The experiments were carried out on healthy male and female rabbits and rats. Rabbits with an average weight of 1850-2550 g were divided into two groups. Every three days, the first group of 24 received and injection into an ear vein of 20-24 mg. of pure silicic acid as a 0.25 or 0.5% sterile colloidal solution. The solution was prepared by dissolving the amorphous silicon dioxide in NaOH and neutralizing with HCl to form a solution isotonic with physiological saline. The injections were given for three weeks, so that the total amount of silicic acid injected was 250-300 mg.

The 14 control rabbits received injections of sterile physiological saline. One week after the last injection had been given, blood was taken from both groups by intracardia puncture. The edema of the ear which in some of the rabbits had developed at the time of the first injection had completely disappeared.

During the experiment, 9 of the rabbits died, including 8 which had received the silicic acid, and 1 of the control group.

Rats weighing 100-175 g were divided into 6 groups. The first group consisting of 8 rats received an injection into the tail of 1.5 ml of a 0.25 % solution of silicic acid, containing 25-26 mg of solid. Four days later, the animals were killed by cutting the carotid arteries, and after the normal treatment, the blood serum was studied polarographically. The five rats comprising the second group received an injection of 50 mg of silicic acid in the form of 1 ml of a sterile 0.5% colloidal solution. They were killed after seven days. Eight of the rats of the third group received an intraperitoneal injection of 1 ml of a 1% highly dispersed suspension of amorphous silicon dioxide (Aéorsil). They were killed after seven days. Rats of the fourth, fifth, and sixth groups served as controls. They received corresponding intravenous or intraperitoneal injections of physiological saline.

The rats of the first group which had been given an intravenous injection showed signs of acute inflammation, while those of the second and third groups who had been given the injection intraperitoneally showed no changes visible to the naked eye.

The polarographic measurements were made by a modification of the method of Brdichek [3, 5]. To 0.4 ml of fresh serum was added 0.1 ml of 0.1 N KOH; it was left to stand for 45 minutes at room temperature, the proteins were then precipitated with a 20 % solution of sulphosalicic acid, and after 10 minutes the solution was filtered.

Into the vessels for the electrolyte of the polarograph were poured 5 ml of the main solution (0.001 N $CaCl_2$, 0.1 N NH_4Cl, and 1 N NH_3), and then 0.25 ml of protein-free serum filtrate was added to each. The polarogram was taken in an atmosphere of hydrogen with a galvanometer of sensitivity 1/150 for rabbit serum, and 1/300 for rat serum. In evaluating the results, account was taken of the distance (in mm) between the cobalt wave and the second catalytic wave. To eliminate errors, polarograms were taken simultaneously for the control and experimental groups. The statistical method of Student was used to evaluate the results.

TABLE 1. Height of Polarographic Threshold of Rabbit Serum (in mm)

Number of experiment	Control animals	Experimental animals (intravenous injection of H_2SiO_3)
1	29	38
2	36	68
3	30	68
4	27	26
5	45	54
6	28	48
7	20	58
8	19	53
9	26	47
10	19	63
11	28	50
12	33	37
13	27	63
14		46
15		26
16		24
Mean	28,23	48,06
S_x	±1,98	±3,64
t	2,8	
P	<2%	

EXPERIMENTAL RESULTS

In the 16 rabbits treated with silicic acid, the average polarographic threshold height was 48.06 mm, and in controls it was 28.23 mm. The difference is highly significant statistically, and indicates that the activity of the serum of the experimental group was 70.24% higher than that of the controls. The results are shown in Table 1.

In the first group of rats, the height of the polarographic wave was 61.12 mm, and in the controls 43.12 mm, i.e. the polarographic activity of the rats which had received the silicic acid into the tail vein was 41.7 % higher than that in the control group.

In the second group of rats, the height of the polarographic wave was 46.6 mm, and it was 36.2 mm in the controls, a difference of 28.8%

In rats of the third group which received a highly dispersed suspension of silicon dioxide intravenously, the height of the polarographic wave was 56.8 mm, and in the controls it was 36.62 mm, a difference of 55.5%

Until now there has been no agreed opinion as to what factors determine the activity of the protein-free serum filtrate. Brdicka [5] attributes great importance to the protein disintegration products which are soluble in sulphosalicylic acid. It is now generally agreed that the shape of the polarogram is determined primarily by the level of the serum mucoproteins [14]. It is important to take this factor into account in both clinical and experimental

TABLE 2.

No. of expt.	Control animals	Exptl. animals (intravenous injection of H_2SiO_3)	Control animals	Exptl. animals (intraperitoneal injection of H_2SiO_3)	Control animals	Exptl. animals (intraperitoneal injection of SiO_2)
1	56	60	36	39	36	56
2	37	57	33	40	33	67
3	62	64	30	40	30	58
4	32	49	20	59	20	45
5	57	64	62	55	62	53
6	29	64			55	60
7	40	68			30	60
8	32	63			27	56
Mean	43,12	61,12	36,20	46,60	36,62	56,87
S_x	±4,64	±2,07	±6,98	±4,29	±5,09	±6,68
t	3,546		1,267		2,41	
P	<1%		<30%		<5%	

silicosis, all the more because, as many authors have shown [9, 11, 13], the mucoprotein level is raised in silicosis. Theobald [12], P. A. Rozenberg and L. A. Zorina [2], and Arato-Sugar [3], have demonstrated a change in the amount of protein disintegration products and amino acids in silicosis.

The depolymerization of mucoproteins of various tissues under the influence of silicic acid is a probable cause of the increased serum mucoprotein. The process takes place in the presence of cysteine, whose increased amount has a great influence on the height of the catalytic wave of the polarogram [5]. Bergstermann [4] considers the height of the level of mucoproteins in the serum as a non-specific protective reaction of the organism against the injection of a foreign body. The fact that the polarographic wave reamined high in rabbits, even after all inflammation had subsided indicates that the increase in height of the catalytic wave in silicosis is a constant phenomenon.

Our results have demonstrated that the injection of silicic acid alone or of amorphous silicon dioxide may by itself induce the polarographic changes which are always encountered in silicosis, and our results therefore support the chemical theory of the etiology of silicosis.

SUMMARY

Studies were made of rabbits and rats injected with silicon compounds.

The polarographic threshold of rabbits receiving intravenous injections of colloidal silicic acid was more than 70.24 % higher than that in control animals receiving physiological saline. As compared to the polarographic wave in the control animals, the wave in the experimental rats was higher (by 41.7% in a group with intravenous injection of silicic acid, by 28.8 % in rats with intraperitoneally administered silicic acid, and by 55.5 % in a group with intraperitoneally injected SiO_2).

These results support the chemical theory of the pathogenesis of silicosis.

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