

THE ACTION OF SODIUM FORMALDEHYDE SULPHOXYLATE IN EXPERIMENTAL PNEUMOCOCCUS INFECTION

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Introduction

Sodium formaldehyde sulphonylate, recently reported upon by Rosenthal (1) for use in mercurial poisoning, has subsequently been studied by the same author (2) as a chemotherapeutic agent in experimental pneumococcus infection.

This chemical is a powerful reducing agent, and on injection confers this property on the body fluids. The toxicity is quite low and large subcutaneous or intravenous doses can be administered with impunity. Its use in bichloride poisoning rests on a good theoretical basis and the practical results thus far lend encouragement to its further anti-mercurial use.

In his infection experiments, Rosenthal used pneumococci of moderate virulence and mice. Sulphonylate was administered either coincident with or three or eight hours subsequent to the injection of pneumococci. Briefly his results showed that when administered early in infections with moderately virulent pneumococci, sulphonylate had considerable therapeutic action. When the use of the drug was delayed until three or eight hours after infection, this action became less striking. Also, when pneumococci of high virulence (i.e., cultures maintained by mouse passage) are used, the curative action of sulphonylate was stated to be much less than that observed with pneumococci of moderate virulence. As contrasted to the chemotherapeutic effectiveness as described, Rosenthal showed that sulphonylate has no *in vitro* antipneumococcal effect.

It appeared of interest to subject sodium formaldehyde sulphonylate to further chemotherapeutic tests utilizing experimental infection of rabbits with pneumococci (3) and delay the beginning of treatment until sixteen to twenty-four hours after infection. It is difficult to imagine pneumonia treatment being instituted quite this early in the course of the disease and burden of proof as to effectiveness of the drug should at least be approximately that exhibited by antipneumococcus serum (4).

Experimental

Rosenthal showed that as much as 2 or 2.5 grams of sulphonylate (in the form of 10 per cent solution) per kilogram of body weight could be well tolerated by mice. In a preliminary way we treated three normal rabbits with 0.5, 1.0, and 2.0 grams sulphonylate per kilogram, intravenously, and all three of these lived. In view of these results the rabbit therapeutic dose decided upon unless otherwise stated was 1.0 gram per kilogram of body weight, and treatments were administered daily up to three days provided the animals survived that long.

The type of experimental pneumococcus infection which we used

TABLE 1. Pneumococcus Infected Rabbits Treated with Sodium Formaldehyde Sulphoxylate Intravenously.

Rabbit Number	Normal Weight and Temperature	Pneumo Type II Culture and Intra-dermally 8-20-34	Examination and treatment subsequently															Remarks
			18 hours			2 days			3 days			4 days			7 days			
			Blood Culture	Temperature	Weight	Sulphoxylate (gms.) per kilo	Blood culture	Temperature	Weight	Sulphoxylate (gms.) per kilo	Blood culture	Temperature	Weight	Blood culture	Temperature	Weight		
2704	1850-103	0.001 cc.	+	105.8	1740	1	+	107.2	1615	1	+	106.4	1600	1	+	106.2	1470	Dead 5 days
2905	1830-103.2	0.001 cc.	+	105.	1730	1	+	104.6	1690	1	+	105.	1660	1	+	105.8	1670	Living at 18 days
2130	1950-103	0.001 cc.	+	105.	1890	1	+	104.4	1790	1	+	106.4	1760	1	+	104.8	1750	Living at 18 days
2763	1900-103.4	0.001 cc.	+	106.	1800	1	+	105.4	1650	1	+	105.4	1600	1	+	105.2	1530	Dead 5 days
2548	1870-102.4	0.001 cc.	+	105.6	1720	1	+	106.2	1600	0	+	106.4	1650	0	+	107.2	1655	Dead 1 day
2112	1880-103	0.001 cc.	+	106.	1520	0	+	106.2	1600	0	+	105.2	1700	0	+	105.4	1700	Living at 18 days
2045	1680-103.4	0.001 cc.	+	106.2	1650	0	+	105.1	1650	0	+	105.2	1700	0	+	103.	1720	Bl. cult. + at 10 days,
2134	1690-102.8	0.001 cc.	+	104.8	1630	0	+	105.8	1730	0	+	105.	1530	0	+	105.8	1850	Dead 22 days
2972	1800-102.8	0.001 cc.	+	105.	1730	0	+	105.8	1650	0	+	106.2	1590	0	+	105.8	1850	Dead 8 days
2968	2120-103	0.001 cc.	+	105.6	2000	0	+	106.	1915	0	+	106.2	1900	0	+	105.6	1540	Dead 5 days
2110	1750-103.4	0.001 cc.	+	105.6	1620	0	+	105.6	1600	0	+	105.4	1600	0	+	103.	1700	Dead 12 days
2584	1980-104	0.001 cc.	+	105.8	1900	0	+	106.6	1750	0	+	105.4	1700	0	+	105.2	1620	Dead 5 days

Although eight additional examinations (weight, temperature, and in some instances blood cultures) were made between the seventh and eighteenth days, they are not recorded here due to lack of space. Significant results are briefly referred to in the text.

has been described heretofore.⁴ Briefly an injection of 0.001 cubic centimeter of virulent mouse-passaged pneumococcus culture intradermally into rabbits results in an early local edematous lesion, positive blood culture, high temperature plateau, progressive loss of weight, and generally death in two to six days. In our experience we have been able to cure a large proportion of such infected rabbits with antipneumococcus serum, provided this is given within twenty-four hours after the pneumococci are injected. While antipneumococcus serum is not uniformly successful in such experiments, we know of no other therapeutic agent closely approaching it in effectiveness, hence sulphonylate if effective would be of much interest.

In the first experiment as shown in Table 1, rabbits, following normal weight and temperature readings, were infected intradermally with 0.001 cubic centimeter of pneumococcus Type II culture heretofore described.⁴ Subsequent events proved this particular infection to be less uniformly fatal than is usual; however, this placed less burden upon the sulphonylate treatment in this case. Eighteen hours subsequent to infection, weight, temperature, and blood culture of these rabbits were taken, and six were treated intravenously with sulphonylate while the remaining six served as controls. Four of the six treated rabbits received 1 gram sulphonylate per kilogram of body weight, while two received 2 grams sulphonylate per kilogram of body weight. These two latter rabbits died in the course of an hour and therefore this larger dose was not repeated in further animals. The four rabbits on treatment with 1 gram sulphonylate per kilogram of body weight each received a full series of three daily treatments. Two of these died on the fourth day, while two recovered between the fourth and sixth days and were entirely normal up to the eighteenth day, which was as long as they were observed. The total mortality in this treated group therefore was 66 per cent, as judged by all deaths, and 50 per cent as judged by deaths among rabbits treated with 1 gram of sulphonylate per kilogram.

In the six infected but untreated control rabbits in this first experiment, two died on the fifth day, a third died on the eighth day, and a fourth, following a stormy temperature course and presenting five positive blood cultures out of six tests, died on the twelfth day. A fifth rabbit, which during the early part of the disease showed temperatures as high as 105.4° F., but no positive blood cultures during this time, appeared to be recovered by the eighth day. On the tenth day, however, a positive blood culture was obtained with a temperature of 103.4°. The temperature again subsided and on the eighteenth day the animal appeared normal. On the twenty-second day, however, this rabbit died, and unfortunately no autopsy or cultures were obtained.

In the past we have observed two sudden deaths in old apparently recovered pneumococcal rabbits, the cause of death as determined at autopsy being due to pulmonary embolism caused by huge endocardial vegetations. Curiously, one of these animals had shown sporadic positive blood cultures coincident with rather large amounts of pneumococcus antibody, and it is believed that infrequently a subacute pneumococcal endocarditis may appear following the acute stages of such infections, and embolic rather than septicemic deaths surely follow. Whether the death of the fifth rabbit in this control group of six animals was brought

about in this way is not known. If this death is not counted, the mortality in the control group was 66 per cent, and quite similar to that in the sulphoxylate-treated group. If the fifth death is counted as pneumococcal, the control mortality was 83 per cent, thus exceeding the mortality of the treated group somewhat.

In this experiment dealing with an infection certainly of less than 100 per cent mortality, the "1 gram per kilo" sulphoxylate treatment started eighteen hours after the injection of pneumococci appears to have questionable merit. The "2 grams per kilo" sulphoxylate treatment appears too severe to use.

At this point one might conclude that sulphoxylate would merit further experimentation, and this was promptly done as follows.

In the second experiment as shown in Table 2, eight rabbits, following normal weight and temperature readings, were infected with 0.001 cubic centimeter of pneumococcus Type II culture. Eighteen hours subsequently, four of these infected rabbits were treated with 1 gram sodium formaldehyde sulphoxylate intravenously per kilo of body weight following routine temperature and weight readings and preparation of blood cultures. On the next day, one rabbit had died and examination and sulphoxylate treatment were repeated on the remaining three. During the day all three of these treated rabbits died.

Of the four infected but untreated control rabbits in this second experiment, one died on the third day and three died on the fourth day. All four showed temperatures up to 106° F. and two or more positive blood cultures.

This infection in the second experiment was 100 per cent fatal as judged by four controls, and sulphoxylate treatment appears not only to have been ineffective but perhaps to have hastened the death of the treated rabbits. Since only eight rabbits were used in the second experiment, it appeared desirable to add to this number in order that the results might be more reliable. Therefore an additional experiment was done as follows.

In this third experiment as shown in Table 3, five rabbits, following the routine preliminary examination, were injected intradermally with 0.001 cubic centimeter virulent Type II pneumococcus culture. On the next day, eighteen hours subsequent to infection, and following the routine examination, three rabbits were treated with 1 gram sulphoxylate per kilogram of body weight. Sulphoxylate in this experiment was administered subcutaneously instead of intravenously. On the second day one of these three rabbits had died, and the other two were again given the routine examination and a second sulphoxylate treatment. Later in the second day these two rabbits died. All three treated rabbits had exhibited temperature up to 105° F. and one or more positive blood cultures.

Two infected but untreated control rabbits in this third experiment, after exhibiting temperatures up to 105.6° F. and one or more positive blood cultures, died on the second and fourth day respectively. This third experiment is similar to the second in degree of virulence of the infection. Since both treated and untreated rabbits died, and the former on the average sooner than the latter, the use of sodium formaldehyde sulphoxylate does not appear promising.

TABLE 2. *Pneumococcus* Infected Rabbits Treated with Sodium Formaldehyde Sulphoxylate Intravenously.

Examination and Treatment Subsequently														
Rabbit Number	Normal Weight and Temperature	Pneumo Type II Culture Intradermally 9-5-34	18 hours				2 days				3 days			Remarks
			Blood culture	Temperature	Weight	Sulphoxylate (gms.) per kilo	Blood culture	Temperature	Weight	Sulphoxylate (gms.) per kilo	Blood culture	Temperature	Weight	
2906	1540—103	0.001 cc.	++	104.	1530	1	++	105.6	1460	1	++	105.6	2100	Dead 1 day
2354	1610—103	0.001 cc.	++	105.4	1550	1	++	103.6	1480	1	++	103.6	2050	Dead 2 days
2726	1740—102.8	0.001 cc.	++	104.4	1700	1	++	105.1	1770	0	++	103.4	1760	Dead 2 days
2353	1930—102	0.001 cc.	++	104.8	1930	0	++	107.	2230	0	++	104.4		Dead 4 days
2635	2240—102.4	0.001 cc.	++	105.	2200	0	++	106.	2090	0	++			Dead 4 days
3786	2160—102.8	0.001 cc.	++	105.	2130	0	++	106.2	1860	0	++			Dead 4 days
2901	2050—103.3	0.001 cc.	++	105.2	1930	0	++		1790	0	++			Dead 4 days
4572	1980—102.2	0.001 cc.	++	106.4	1900	0	++				++			Dead 3 days

TABLE 3. Pneumococcus Infected Rabbits Treated with Sodium Formaldehyde Sulphoxylate Intravenously.

Examination and Treatment Subsequently															
Rabbit Number	Normal Weight and Temperature	Pneumo Type II Culture Intradermally 9-10-34	18 hours						2 days				3 days		Remarks
			Blood culture	Temperature	Weight	Sulphoxylate (gms.) per kilo	Blood culture	Temperature	Weight	Sulphoxylate (gms.) per kilo	Blood culture	Temperature	Weight		
3270	2020—101.4	0.001 cc.	+	105.	1820	1	+	101.	1730	1	+	105.	1800	Dead 2½ days	
3271	2160—102.	0.001 cc.	+	105.2	1940	1	+	103.	1780	1	+	105.		Dead 2½ days	
3272	1860—101.6	0.001 cc.	+	101.4	1880	1	+				+	105.8		Dead 2 days	
3274	2010—102.8	0.001 cc.	++	105.6	1930	0	++	102.4	1720	0	++			Dead 2½ days	
3275	2000—103.	0.001 cc.	++	106.2	1900	0	++	105.8	1850	0	++	105.	1800	Dead 4 days	

Discussion

We have tested sulphonylate as a chemotherapeutic agent in rabbit pneumococcus infection and have probably subjected the chemical to more severe test than did Rosenthal. We used pneumococci regularly subjected to animal passage, and of high virulence. Although Rosenthal used "virulent culture" in his detailed experiments, it appears such culture was not passaged, and this author stated in a footnote that when highly virulent passaged pneumococci were used in experimental infections the therapeutic action of sulphonylate was not so pronounced. In addition to our use of highly virulent culture, we began sulphonylate treatment at a time in the disease during which antipneumococcus serum has been shown to be effective, i.e., eighteen hours after infection, whereas Rosenthal started sulphonylate treatment either at the same time as the infection was administered or three hours or eight hours subsequently.

It is well known that while experimental pneumococcus infection in mice or rabbits is rather easy to prevent, it is difficult to cure, and with each succeeding hour of time elapsing between infection and attempted curative treatment this difficulty becomes greater. Active immunity against the pneumococcus begins to appear in four or five days following injection of vaccine, hence successful prophylactic vaccine must be administered at least this long before infection is attempted. Real therapeutic action should preferably be attempted sometime following infection, and the later such therapeutic agent is found useful, the more promise it would hold.

Our main purpose in these experiments, therefore, was to find whether sulphonylate was as good as or better than immune serum in virulent pneumococcus infections in which a doubly severe test of curative action is placed on the therapeutic agent.

It appears from our results that sulphonylate is not of marked usefulness in pneumococcal infection.

Conclusions

1. Sodium formaldehyde sulphonylate has been subjected in rather severe tests to chemotherapeutic trial in virulent pneumococcus infections in rabbits.
2. Sulphonylate doses used were mainly intravenous and mainly 1 gram per kilogram of body weight. A series of three daily doses was administered to all treated rabbits surviving long enough for this.
3. Sulphonylate did not appear definitely to help in pneumococcus rabbit infection.

NOTE: Our thanks are due Mr. James Moss for his collaboration in the experiments herein reported.

References

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