The Human Toxicology Of Dimethyl Sulfoxide

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INTRODUCTION

On November 11, 1965, research on DMSO in the United States came to an abrupt halt. A conference between the Food and Drug Administration and the pharmaceutical companies who were involved in the research was called because lens changes had been observed in a number of mammalian species. No changes ha been observed in man or any primates. The FDA and the pharmaceutical companies agreed, because there had been no pretreatment examinations of eyes and a large number of patients were under therapy, to discontinue the clinical studies: Somehow, at this time, DMSO gained a reputation of extreme toxicity, comparable to that of thalidomide and some other drugs that had previously run into major toxicology problems. Many of us in the pharmaceutical industry felt that this reputation was undeserved.

A refractive index change in the lens (not an opacity) had been observed after ? months at a dose of approximately 5 g/kg in dogs, rabbits, and pigs. No microscopic or chemical differences could be found between the lenses of the treated animals am the controls. In the affected animals, there appeared two distinct zones of different refraction. This could easily by observed with an ophthalmoscope and with the slit lamp. It appeared to be a dose-related effect, and it diminished as the dose was reduced. It is noteworthy that the effect was produced at 50 to l00 times the usual human therapeutic dose.

In November, 1965 there had been no cases of confirmed eye damage or significant complaints in the studies of any of the pharmaceutical firms. Pre-treatment examinations of eyes had not been performed. We all felt that to re-examine all the patients who had been under treatment at this stage would be fruitless exercise, because of the age of many of the patients and their preexisting eye problems. We elected, therefore, to check certain long-term patients on high doses. Drs. Jacob and Rosenbaum, in Portland, Oregon, had 32 patients examined by ophthalmologists connected with the University of Oregon Medical School. These had been treated for from 3 to 19 months, at an average dose of 30 g DMSO per day. None of these showed any of the characteristic lens changes that had been seen in the animals. One patient in Seattle was thoroughly checked. He had by chance had a complete pretreatment examination performed by an ophthalmologist several months prior to his neck injury. He was 19 years old, and at the date of his post-treatment exam he had received 60 g DMSO per day for 20 months. His follow-up exam was completely negative. This included tonometry, visual field, refraction, and slit lamp examination.

Dr. Scherbel, at Cleveland Clinic, had under treatment 44 cases of scleroderma. Their treatment was still continued under the new FDA rules. Some of these patients had received as much as 3 g/kg per day. Some were treated for as long as 23 months. Many lens abnormalities were observed in this group of patients, but none of those characteristically observed in the DMSO-treated animals. Therefore, the results of the examination of scleroderma cases were somewhat inconclusive.

During 1966, the pharmaceutical companies continued to collect case reports and no real toxicity of any kind was being observed. Merck and Company gradually collected 17,000 cases. Syntex collected approximately 7,00G and E. R. Squibb and Sons around 3,000. Monkey studies continued both in Germany and in the United States. No lens changes were observed at 11 g/kg dermally and 5 g/kg orally per day after one year. We came to the conclusion that these lens changes were probably species-specific, and that the primate was probably much more resistant than other mammals.

In January, 1967, I was retained as a consultant at E. R. Squibb and Sons to develop a program to reestablish clinical research on DMSO. It was apparent that the first step, before the contemplated studies in acute trauma and acute inflammation, would be a thorough study in human toxicology. We needed to determine the real degree of toxicity of the compound. If the material was truly toxic, no company would want to subject patients to risk; but if it could be proved clean, the rather wide potential uses of DMSO would warrant such a toxicology study. The Food and Drug Administration at the same time had planned a short-term study to evaluate only the lens problem. They agreed after consultation with E. R. Squibb and Sons to include their study as part of a complete toxicology study.

The short-term study was conducted at the State Prison Hospital at Vacaville, California in October of 1967. The long-term study was conducted at the same institution from November 21, 1967 to February 20, 1968. The chief investigator was Charles Lebo, M.D. A large number of other physicians became involved in the project because of the specialized studies needed, and because of the careful supervision and management essential in such a complex and difficult study. It is interesting to note that Mr. Urban, the administrator of the Solano Institute, told us that this was the most thorough and comprehensive toxicology study that they had ever conducted.

Ophthalmologic examinations were performed by Frank Hull, M.D., of Fairfield, California. We needed the nearly full-time services of a board-qualified ophthalmologist who had the necessary interest to conduct this part of the study. He was assisted by Donald Wood, Ph.D., from Portland, Oregon, who had a group of animals that had been treated with DMSO. He brought these down to Fairfield, California so that Dr. Hull could examine them and be well acquainted with the characteristic lens changes at various doses.

Bone-marrow studies were performed and interpreted by R. Wallerstein, M.D. of San Francisco. Neurological studies, including electroencephalograms, were done by R. E. Cook, M.D., of San Francisco, and complete routine physical checkups were performed by the attending medical staff of the Solano Institute of Vacaville, California.

Pulmonary function studies and electrocardiograph interpretations were done by R. D. Brobyn, M.D. In addition, the general supervision of the administration of the drug and monitoring of the results on an almost daily basis were done by Dr. Brobyn. It was necessary to maintain as low a dropout rate as possible in such a difficult study, as we expected considerable skin reaction and breath odor, and knew that we would get a lot of complaints from the prisoners and the people with whom they came into contact. Analysis of the blood and urine specimens and the

computer printout of the lab data were the responsibility of the United Medical Laboratories of Portland, Oregon. This was necessary because of the massive amount of laboratory information that was being collected. The study was conducted with 80% DMSO gel at 1 g/kg per day, by daily dermal application. This was estimated to be 3 to 30 times the human treatment dose. A call for volunteers was made at the institution. We asked for perfectly healthy subjects between the ages of 21 and 55. Approximately 400 responded; of these, 213 quality by history. It was necessary that they should have no preexisting ophthalmologic, pulmonary, cardiac, hepatic, renal, or hematologic diseases. These men were given a complete physical examination, including ophthalmologic tests by Dr. Hull. He performed initial laboratory studies to ensure that their baseline values were within the normal limits. Essentially, we selected individuals with a good past medical history and normal complete examinations for the study. They had to be sufficiently motivated to participate in a difficult and possibly uncomfortable study for up to three months. Unreliable and emotionally unstable individuals were excluded, to minimize distortion of the side effects.

The first phase of the study was for 14 days. Group A consisted of 78 subjects who would receive the DMSO. Group B, which consisted of 33 subjects, would act as controls. We expected some dropout rate from Group A because of skin reaction, local side effects, and rather bad breath odor, but we hoped that eventually 66 active subjects would complete the study. This meant that we would have two treated individuals for each contro1. Group A eventuality consisted of 65 subjects.

Each subject was weighed in kgs, and this was multiplied by 100/80 to compensate for the 80% concentration. An average of 15% was added, and this amount was dispensed in a cardboard container. The entire dose was applied in multiple layers and rubbed into the skin until it was completely absorbed. The container had to be returned to the inspecting official to make sure that every bit had been used. This was carefully monitored every day on all subjects. Complete absorption required up to 2 hours, and each man was checked daily before leaving the administration area (before he could apply any clothes). One dose per day was used in the evening for convenience after a shower at the end of the work day. Only one washing was allowed per day, except of the hands. No dermatologic preparations of any kind were to be used, because this would distort the skin effect and possibly alter the absorption rate. The DMSO was used daily for the full 14 days by the 65 subjects. All of the 13 dropouts occurred after the first 1 or 2 applications, due to local skin effects.

Blood and urine samples were obtained from all subjects 7 and 14 days after the initiation of treatment. At the end of the 14-day period, all subjects received a complete examination identical to the pretreatment examination, plus a complete ophthalmologic examination. A lens exam with the slit lamp was repeated at the 4 week point, or 2 weeks after treatment. A complete ophthalmologic exam was done after 6 weeks, or 4 weeks after cessation of therapy. All subjective side effects were reported in the DMSO-treated subjects, or all of Group A. Unfortunately Group B was not specifically questioned, and only volunteered complaints. It is interesting to note that there were no serious complaints except those of histamine effects and local effects. The physical examinations covered blood pressure, pulse, temperature respiration, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, abdomen, liver, kidney, spleen, external genitourinary and rectal system,

extremities, skin, and neurology.

The ophthalmologic examination consisted of examination with the slit lamp and the ophthalmoscope, and a complete testing of refraction, tonometry, and visual fields.

Laboratory examinations in this group consisted of a complete blood count with indices, evaluation of platelets, and reticulocyte counts, UA and chemistries: BUN, creatinine, FBS, cholesterol, thymol turbidity, total protein, albumin, globulin, A/6 ratio, uric acid, alkaline phosphatase, SGOT, SGPT, and PBI.

Specimens were collected on Monday and Tuesday of each week. There were two treatment groups for the study. These were sent to United Medical Labs by direct flight from Sacramento to Portland. All abnormal results were reported to the institute, and also to Dr. Brobyn, by Wednesday. Recheck of abnormal values and the drawing of repeat specimens were set up for Thursday and Friday. This was according to the protocol. If the repeat value was within normal limits, the first value was rejected and was not entered on the final reports. It was retained in the original lab printouts, which were also submitted to the Food and Drug Administration. In practice, a complete profile was always drawn for the repeat study. The original sample and the repeat run were in duplicate, and a third sample was retained to be run through the autoanalyser if there was an excessive difference between the first two, in order to obtain maximum reliable results.

RESULTS

Laboratory Examinations

The analysis of the battery of blood and urine studies indicated that the daily doses of 1 g DMSO/kg for 14 days produced no toxicity. There were some scattered and transient abnormal values in both the treated and the control groups. With the single exception of peripheral eosinophil counts, however, there was no indication that such abnormal values were any more frequent in the DMSO group than in the control group. One subject exhibited a BUN elevation from 14 to 24 after 2 weeks of application of DMSO; otherwise, the BUN values remained essentially within normal limits. Another subject exhibited SGOT elevation from 17 to 53 after 1 week of therapy. His other liver function tests remained within normal limits. One of the control subjects had an SGPT elevation from l4 to 350 after l week of observation. A single subject who was receiving DMSO exhibited an increase in his FBS from 78 to 122.

Ten of the 65 subjects who were receiving DMSO exhibited an eosinophilia. No such eosinophilia occurred in the control group. In only one instance was the level greater than 10. An eosinophilia of 23 was observed. This was not unexpected, because of the cutaneous histamine-releasing effect of DMSO. Another interesting but unexplained observation was the appearance of an occasional large lymphocyte, with deeply basophilic cytopiasm. This occurred in 10 Group A subjects and 1 Group B subject. It is noteworthy, however, that they were also observed in the 90-day human studies and here they occurred with equal frequency in both the DMSO-treated and control groups. I concluded that this was some viral infection prevalent in the institution, and was not caused by the DMSO.

Ophthalmologic Examination

The results of the slit lamp examination, ophthalmoscopy, and determination of visual fields and refraction were totally reassuring. There were no significant changes during the 14-day study, nor were there any alterations in the post-treatment examinations. There were no changes in any way suggestive of an abnormal refractive index characteristic of the changes observed in animals. There was no change in the intraocular pressure in either the treated or the control subjects. Some patients experienced a vague conjunctival irritation, but this may have been caused by some DMSO inadvertently rubbed in or around the eyes.

Physical Examinations

The complete physical examinations did not indicate any significant abnormalities. The findings were identical to those of the pretreatment examinations except for a variable degree of skin reaction, which was characteristic of the type served in all of the clinical trials. This consisted of wheal and erythema, drying and scaling. All of the reacting skin completely returned to normal within 3 weeks of treatment. The systolic blood pressure was somewhat lower in a few subjects in the DMSO-treated group. I concluded that this was because of increased cutaneous blood flow from the wheal and erythema phenomenon and local histamine released.

Systemic Side Effects

The daily administration of l g/kg of DMSO was associated with some sedative headache, nausea, and dizziness (TABLE I). No serious side effects were observed.

90-DAY TOXICOLOGY STUDY

A smaller group of subjects were qualified according to the same criteria as was used in the 14-day study, They knew that they would use the drug for 3 months and be subjected to many more toxicity studies. Higher motivation was essential. Group C was to receive 80% DMSO gel at 1 g/kg. Group D would serve as controls and receive no medications.

TABLE I
SIDE EFFECTS OF 80% DMSO GEL IN 78-SUBJECT STUDY *

Side Effect	Number of Subjects	Percent Incidence
Sedation	34	52
Headache	27	42
Nausea	21	32
Dizziness	12	18
Burning or aching eyes	6	9
Vomiting	4	6
Xerostomia	3	5
Influenza-like	3	5
syndrome	3	5

Diarrhea	3	5
Weight gain	3	5
Weight loss	2	3
Constipation	2	3
Dry nasal passages	1	2
Dyspnea	1	2
Dry throat	1	2
Sore throat	1	2
Cough Frequency of urination	1	2
Anorexia	1	2

*The dose was 1.0 g DMSO/kg daily. Because 13 subjects were dropped from the study for reasons other than the side effects listed in this table, the percentages indicated in the incidence column are based on a total of 65 subjects.

Fifty-four subjects were selected for Group C; 26 subjects were selected for Group D.

During the study 14 subjects dropped out from Group C. Twelve of these stopped in the first few days because of skin reaction. Two dropped out at days 31 and 52 respectively, for breath odor and personal reasons. Thus 40 subjects completed the 90-day use of 80% DMSO gel at I g/kg. There were no losses in the control Group D.

Each group was further divided, into C1 and C2 and D1 and D2 respectively. C1 and D1 received the standard laboratory tests of the protocol. Groups C2 and D2 received special tests. There were 8 subjects in C2 and 5 in D2.

The dosage and administration of the DMSO was exactly as described in the 14-day study.

Method of Study

Physical, ophthalmological, and laboratory examinations were performed prior to the start of the study. All subjects who received DMSO started medication on November 20, 1967, and they finished on February 18, 1968 if the full 90-day course was completed. Blood and urine samples were obtained from all subjects at l, 2, 4, 6, 8, and 13 weeks (2/20/68). All subjects received a physical examination at the end of the study (2/22/68). Ophthalmological examinations were conducted weekly up to the twelfth week, and at the fourth, sixth, ninth, twelfth, and eighteenth month of the study.

Physical examinations consisted of determinations of blood pressure, pulse, temperature, and respiratory rate, examination of the neck and thyroid, lungs, heart, liver, kidney, spleen, abdomen, genitourinary system, rectum, and extremities, and an E.N.T. and neurological examination.

Ophthalmological examinations included slit lamp examinations, ophthalmoscopy,

and tests of refraction, tonometry, and tangent field. (The results of these examinations are available on individual report forms for each subject.)

Laboratory examinations were handled in the same fashion as in the 14-day study. They included hematology (rbc, wbc, differential count, hemoglobin, hematocrit, morphology, color and saturation indices, platelets, and reticulocyte count): urinalysis; blood chemistries (creatinine, glucose, [fasting], BUN, cholesterol, thymol turbidity, total protein, prothrombin, albumin, globulin, A/G ratio, uric acid, alkaline phosphatase, SGOT, SGPT, PBI, PSP, creatinine clearance, bilirubin, BSP, calcium, magnesium, phosphorus, sodium, potassium, chloride, CO" ICD, CPK, urinary hydroxyproline). In addition, Groups C, and D, received cerebrospinal fluid and bone-marrow examinations, and other special examinations.

Acceptable Laboratory Values

The normal range of values for the United Medical Laboratories (UML) is given in parenthesis for each test on the subject's laboratory form. If an abnormal value was observed, the test was repeated. (Actually a group of tests would be repeated, inasmuch as the autoanalyzer was programmed to run a battery of tests simultaneously.) Specimens were taken on a Monday or Tuesday and flown to UML in Portland, and results were returned by phone, usually on Wednesday or Thursday. Repeat specimens were taken a few days later, usually on Friday. If the second (or third) value fell within the normal range, this was assumed to be the correct figure and the previous figure was considered aberrant. If on the repeat, the figure still fell outside the normal range, a further repeat was done, and if the value was still abnormal, the value for that test was considered abnormal and reports such.

RESULTS

Laboratory Examinations

As in the previously reported 14-day study, with the exception of eosinophilia, no significant abnormalities were observed in the large battery of blood chemistries, peripheral blood and urine analyses, or various special procedures. Thus, it is evidence that administration even of massive 1.0 g/kg doses of DMSO daily for as long as 14 days produced no significant toxicity, so far as can be determined by both routine and special laboratory studies.

A transient eosinophilia during the first few weeks of DMSO application occurred in 23 (51%) of the 45 DMSO-treated subjects. Although a similar eosinophilia was noted in 8 (31%) of the 26 control subjects, it is thought that the higher incidence in the DMSO group denotes a true drug effect. It is suggested that this effect is produced by the cutaneous histamine-releasing property of DMSO.

Again, as cited in the 14-day safety study, an occasional large lymphocyte with mottled blue cytoplasm and coarse chromatin was observed for 5 days or more in the peripheral blood of 20 (44%) of the 45 subjects who received DMSO, and also, 11 (42%) of the 26 control subjects. Thus it is unlikely that DMSO is responsible for this altered cell morphology.

There was no evidence of gross blood loss or anemia in the DMSO-treated

subjects. A modest decrease in hematocrit, hemoglobin and/or the red blood cell count occurred in two subjects. The MCV, color index, and volume index increased in three other subjects.

There were no significant abnormalities in the BUN or urinalyses. A decrease of 15-min PSP excretion was observed in 1 of 8 DMSO-treated subjects.

Serum SGOT levels increased (to between 40 and 100) in 3 DMSO-treated subjects. The levels returned to normal while DMSO therapy was continued, in all except one subject. This subject exhibited an SGOT of 78, and an SGPT of 60, at the conclusion of therapy; however, other parameters of the liver functions were normal. It was not possible to obtain further follow-up on this subject. It is noteworthy that 5 of the 26 control subjects exhibited elevated transaminase levels at some time during the study. These abnormalities may relate to the observation that 6% of the sampled population of the prison had elevated transaminase levels.

One subject exhibited increasing concentrations of serum alkaline phosphatase with continuing DMSO treatment; however, al) of his other liver function parameters remained normal.

Unaccountably, BSP retention tests were abnormal in 1 of 8 DMSO-treated subjects and 3 of 5 control subjects.

Although fasting blood glucose levels were sometimes slightly above or below normal limits in a number of the DMSO-treated and control subjects, there was no consistent abnormal trend. A slight hyperglycemia was more frequent in the control group, whereas the incidence of slight hypoglycemia was similar in the two group Glucose tolerance tests were normal in 6 of 8 DMSO-treated subjects so evaluated. The tests were inconclusive in the other 2 subjects. (One of 5 glucose tolerance test conducted among the control subjects was also inconclusive.)

An increased prothrombin time was noted in one DMSO-treated subject. Inasmuch as prothrombin times were determined for all DMSO-treated subjects, it seems unlikely that this one instance was drug-related.

Although some alterations in cerebrospinal fluid protein were observed in the DMSO-treated subjects, there were similar findings in the control group. There were no alterations in the CSF glucose values for either group. It appears that DMSO has no significant effect on the cerebrospinal fluid.

Repeat bone-marrow examinations were carried out on 7 DMSO-treated subjects and 4 control subjects. There were no significant abnormalities.

An attempt to measure urinary hydroxyproline levels in subjects was not successful. The results were quite inconsistent and were cast out on the assay method. Similar laboratory difficulties were encountered in the assessment of urinary hormone assays.

Special Procedures

Pulmonary function studies, as stated previously, were conducted on all subjects, because this was one of the main areas of suspected toxicity. These were done

before treatment and after 2, 4, 6, 8, and 13 weeks. There were no significant changes, although 4 DMSO-treated subjects exhibited slight alterations in the force vital capacity, forced expatory volume, and pulmonary resistance. Among the control subjects, 2 exhibited the same type of changes. There was no evidence of bronchospasm. DMSO treatment had no effect whatsoever on the serial electrocardiograms obtained on the subjects. Serial EEG tracings were obtained on all the subjects in C2 and D2. They were completely normal. Photostimulation and hyperventilation produced no EEG changes. We can conclude that dermal administration of DMSO at l g/kg daily has no effects on the central nervous system.

Ophthalmologic Exams

Slit lamp examinations, ophthalmoscopy, and refraction, tonometry, and visual field tests were done throughout the study as previously described. No abnormalities were noted in any of the treated or control subjects. This appears to be one of the most significant statements that we can make. There appears to be no ocular toxicity from DMSO after 3 months' treatment at this quite high dose.

Side Effects

The skin reaction and breath odor were anticipated and did occur. Numerous other side effects occurred in both the DMSO-treated and control groups. A complete listing of these is provided in Tables 2 and 3. The comparison of the DMSO-treated and control groups suggests that the true side effects of massive doses of DMSO include some sedation and occasional insomnia and nausea. A small amount of dizziness and diarrhea also occurred in the DMSO-treated group.

TABLE 2
SIDE EFFECTS IN THE GROUP TREATED WITH 80% DMSO

Number of Subject (54 in Total)	Side Effects of 1 g DMSO/kg Daily
21	skin reaction, "collapse" (apparently due to severity of skin reaction)
28	dizziness, headache, loose stools
30	sedation, dizziness, nausea, vomiting
31	weight loss
32	sedation, nausea, headache, constipation
71	dizziness, headache, loose stools, eye burning, anal irritation, ache in right hip
82	nausea, headache, skin burning
93	headache, flu, tinnitus, vesicular skin eruption
95	dizziness, pruritus
97	no adverse effects
106	"discomfort" *
112	sedation, headache, loose stools, skin burning, swelling of ankles
113	"discomfort" *
116	no adverse effects

121	"discomfort" *
122	"discomfort" *
123	sedation, vomiting, headache, hazy vision, leg cramps
125	nausea, headache, loose stools, skin irritation
127	nausea, visual fuzziness, decreased libido, pruritus
134	sedation, nausea, headache
135	skin reaction, headache
146	pruritus
150	headache, insomnia, skin burning
152	headache, tinnitus, urinary hesitancy, skin burning
155	sedation, dizziness, headache, insomnia, skin burning
157	sedation, dizziness, nausea, headache, insomnia
160	nausea, vomiting, headache, skin irritation
301	skin irritation
302	breath odor
303	"discomfort" *
304	rhinorrhea, tattoos fading
305	nausea, headache, loose stools, skin irritation, insomnia
306	sedation, nausea, headache
308	"discomfort" *
309	nausea, vomiting, increased libido
310	sedation, headache
311	sedation, nausea, headache, insomnia
312	"discomfort" *
313	dizziness, headache, visual haziness
314	nausea, headache, skin irritation
316	"discomfort" *
318	headache
319	sedation, headache, skin irritation, insomnia
320	nausea, headache, skin irritation, insomnia
321	sedation, headache
322	"excessive pain" *
323	headache, weight loss
324	nausea, depression, irritability
325	sedation, headache
326	nausea, headache, loose stools, dyspnea (pulmonary function OK)
327	sedation, headache, skin irritation, insomnia, nightmares, urinary hesitancy
329	bradycardia, confusion, weakness, skin irritation
330	skin irritation, bloated feeling, insomnia
331	loose stools, skin irritation
*This is assumed to be local cutaneous discomfort.	

TABLE 3
SIDE EFFECTS IN CONTROL GROUP

Subject Number (26 in Total)	Side Effects
40	headache
42	headache
68	headache
73	headache, coryza
75	influenza
85	headache (post-spinal tap)
88	pleurisy
91	coryza
94	headache (post-spinal tap)
103	vomiting, upper respiratory infection
107	coryza
111	sedation, dizziness (after blood withdrawal), headache, earache
120	headache
129	decreased visual acuity
137	ear infection
138	headache
144	headache
145	headache, lower respiratory infection
147	headache (post-spinal tap)
156	headache (post-spinal tap)
158	headache

CONCLUSION

A very extensive toxicology study of DMSO was conducted at 3 to 30 times the usual treatment dose in humans, for 3 months. DMSO appears to be a very safe drug for human administration, and in particular, the lens changes that occur in certain mammalian species do not occur in man under this very high prolonged treatment regimen. I am very glad to be able to present these data at this time, so that we can permanently dispel the myth that DMSO is in any way a toxic or dangerous drug. After considerable work in evaluating thousands of cases that were treated in 1964 and 1965, and after this special toxicology study, I feel that we can unequivocally say that DMSO is quite safe, and now the only necessary task is the proof of its efficacy in specific indications.
