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Effects of dimethyl sulfoxide on the oxidative function of human neutrophils

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Dimethyl sulfoxide (DMSO) has been demonstrated to suppress the in vitro microbicidal activity of neutrophils. In addition, this compound has been described as having significant anti-inflammatory activity. These properties have generally been attributed to the effectiveness of this compound as a hydroxyl radical scavenger. However, DMSO can also act as a reductant under certain conditions, yielding its fully oxidized form, dimethyl sulfone ($DMSO_2$), as the product. Therefore, we evaluated the ability of these two compounds to interfere with the production of oxidants other than the hydroxyl radical by stimulated human neutrophils. In a cell-free assay, DMSO was found to quench the oxidant activity of hypochlorous acid. Neither DMSO nor $DMSO_2$ reacted with superoxide, hydrogen peroxide, taurine chloramine, or monochloramine in this system. However, both DMSO and $DMSO_2$ significantly suppressed the production of superoxide, hydrogen peroxide, and hypochlorous acid by human neutrophils stimulated with either phorbol myristate acetate or opsonized zymosan. Neutrophil viability was not reduced by either DMSO or $DMSO_2$. Inhibition of the oxidative function of stimulated neutrophils by DMSO may provide an alternative explanation for the effects of this compound on the microbicidal activity of neutrophils and as an in vivo anti-inflammatory agent. (J Lab Clin Med 1987;110:91-6)

Abbreviations: DMSO = dimethyl sulfoxide; $DMSO_2$ = dimethyl sulfone; EDTA = ethylenediaminetetraacetic acid; $HOCl/OCl^-$ = hypochlorous acid–hypochlorite ion solutions; H_2O_2 = hydrogen peroxide; NADPH = reduced nicotinamide-adenine dinucleotide phosphate; NaOCl = sodium hypochlorite; NH_2Cl = monochloramine; NH_4Cl = ammonium chloride; O_2^- = superoxide anion; OPD = o-phenylenediamine; OpZy = opsonized zymosan; PBS = phosphate-buffered saline solution; PMA = phorbol myristate acetate; TauCl = taurine chloramine; TNB = 5-thio-2-nitrobenzoic acid

Dimethyl sulfoxide has been reported to be efficacious in the treatment of a variety of inflammatory and rheumatologic diseases.[1-5] This compound's anti-inflammatory effects have generally been ascribed to its ability as a scavenger of hydroxyl radicals, which are purported to be involved in inflammation and tissue damage.[6-9] Neutrophils are a major component of the inflammatory process and have been reported to generate hydroxyl radicals during the respiratory burst,[10,11] although a recent report has suggested that stimulated neutrophils may not produce this species because of an internal quenching mechanism.[12] Repine et al.[13] have demonstrated that in relatively high concentrations (100 to 300 mmol/L), DMSO was able to inhibit the bactericidal activity of neutrophils against *Staphylococcus aureus* and suggested that this effect was the result of the hydroxyl radical scavenging capabilities of DMSO.

Neutrophils generate a variety of reactive species during their oxidative metabolism. These include O_2^-,[14] H_2O_2,[14,15] $HOCl/OCl^-$,[16-20] NH_2Cl,[21] and TauCl.[19,22] among others.[23] The possibility exists that DMSO could

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Table I. Reactivity of DMSO and $DMSO_2$ with products of neutrophil oxidative metabolism

Concentration (mmol/L)	Percent decrease				
	HOCl/OCl^-*	H_2O_2*	NH_2Cl*	TauCl*	O_2^-†
DMSO					
0.1	26.0	—	0.0	—	—
1.0	88.8	0.0	0.0	0.0	0.0
10.0	97.8	0.0	0.0	0.0	0.7
100.0	97.4	0.0	0.0	0.0	0.7
300.0	95.0	0.0	0.0	0.0	0.0
$DMSO_2$					
0.1	9.4	—	0.0	0.0	—
1.0	2.4	0.0	0.0	0.0	0.0
10.0	1.0	0.0	0.0	0.0	0.0
100.0	16.4	0.0	1.0	7.6	0.0
300.0	59.0	0.0	0.0	12.4	0.0

Values are expressed as mean of results from three experiments.
*Percent decrease (of an initial 500 μmol/L concentration) after a 30-minute incubation with DMSO or $DMSO_2$.
†Percent decrease of xanthine-xanthine oxidase–mediated superoxide dismutase–inhibitable cytochrome c reduction in 20 minutes (baseline cytochrome c reduction of 26.6 μmol/L).

interact with these species as well as with hydroxyl radicals. In addition, high concentrations of DMSO might inhibit the formation of these species directly, as well as quenching them after their release into the extracellular milieu. Because DMSO may act as a reductant under certain circumstances, it could have the potential to react with certain oxidants directly, yielding the fully oxidized form of DMSO, $DMSO_2$.[24]

In this study we sought to determine whether DMSO or $DMSO_2$ could react directly with any of the oxidants produced by stimulated human neutrophils in a cell-free system, or whether the compounds could affect the production of these various species by the stimulated cells. The results of our experiments demonstrate that both DMSO and $DMSO_2$ can significantly suppress the production of O_2^-, H_2O_2, and HOCl/OCl^- by stimulated neutrophils, apparently by directly interfering with the oxidative metabolism of the cell, rather than by quenching the oxidants once they are produced.

METHODS

Materials. DMSO, $DMSO_2$, xanthine, xanthine oxidase, 5,5'-dithiobis(2-nitrobenzoic acid), sodium borohydride, superoxide dismutase, cytochrome c (type VI), zymosan, horseradish peroxidase, phenolsulfonphthalein (phenol red), PMA, methionine, taurine, and EDTA were obtained from the Sigma Chemical Co., St. Louis; OPD was obtained from Eastman Kodak Co., Rochester, N.Y., NH_4Cl and H_2O_2 from Fisher Scientific Co., Pittsburgh, and NaOCl from the Aldrich Chemical Co., Milwaukee. To prepare TNB, 5,5'-dithiobis(2-nitrobenzoic acid) was reduced with a twofold molar excess of sodium borohydride; fresh TNB solutions were prepared weekly. PMA was dissolved in DMSO (1 mg/ml) before being diluted in buffer. Zymosan was opsonized by incubating 5 mg/ml in human serum (which was stored at −70° C) at 37° C for 30 minutes followed by washing three times in saline solution. The molarity of the NaOCl solutions wa determined by their absorbance at 292 nm. NH_2Cl was pr pared by incubating NaOCl with a 10-fold excess of NH_4C TauCl was prepared by incubating NaOCl with a 10-fo excess of taurine. Conversion to the chloramine was evaluate under these conditions by UV spectroscopy; in both case the NaOCl peak at 292 nm disappeared, to be replaced b the NH_2Cl peak at 242 nm or the TauCl peak at 252 nm.

Neutrophils. Human peripheral blood neutrophils we isolated by Hypaque-Ficoll density gradient centrifugatio and removal of contaminating red blood cells by hypoton lysis as previously described.[25] The resulting preparations cor sisted of 98% to 99% neutrophils with 95% to 100% viabilit as assessed by trypan blue staining. Experiments were ca ried out in PBS containing 5 mmol/L KCl, 1 mmol/L CaCl 1 mmol/L $MgSO_4$, and 5 mmol/L glucose at pH 7.4.

Quenching assays (HOCl/OCl^-, H_2O_2, NH_2Cl, TauC O_2^-). Quenching of HOCl/OCl^-, NH_2Cl, H_2O_2, and TauC was evaluated by using the oxidation of OPD to determin the remaining oxidant concentration. Standard curves we first prepared by using half-log dilutions of the oxidants. S lutions containing 500 μmol/L concentrations of the oxidan were placed in 12 × 75 mm plastic tubes, to which eith DMSO or $DMSO_2$ were added in 0.1, 1, 10, 100, and 30 mmol/L concentrations; the samples were incubated at 25° for 5 minutes, and then 1 mg/ml of OPD and (for the H_2O assay only) 10^{-3} mg/ml of horseradish peroxidase were adde and the samples incubated at 37° C for another 40 minutes After the incubation, absorption was measured at 420 nm o a Beckman DU-6 spectrophotometer (Beckman Instrument Fullerton, Calif.) and compared with the values on the star dard curves. The quenching assay for O_2^- was performed b adding DMSO or $DMSO_2$ (in the above concentrations) t 1 mmol/L xanthine, 10 U/L xanthine oxidase, and 10 μmol/L cytochrome c with or without the addition of 2 μg/ml superoxide dismutase. The mixture was incubated a 37° C for 20 minutes, and absorbance was measured a 550 nm. The results were converted to nanomoles of cyto

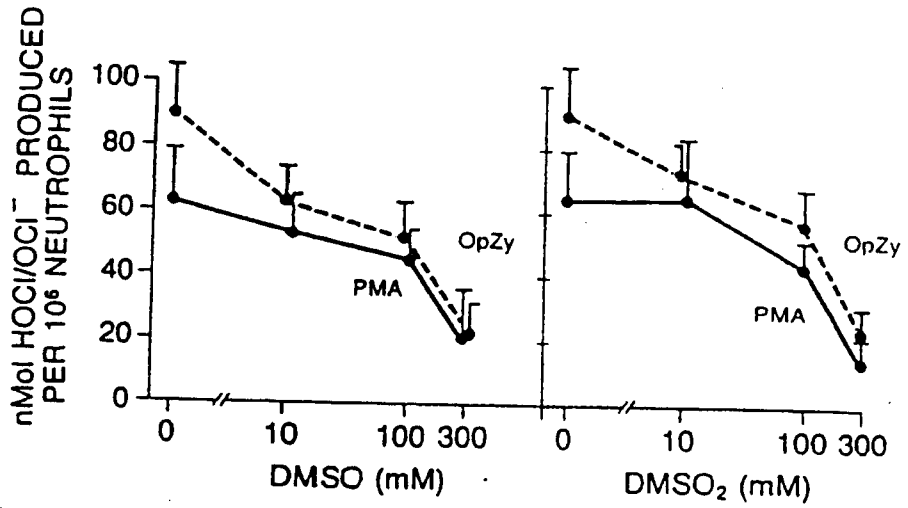

Fig. 1. Effect of DMSO and $DMSO_2$ on production of $HOCl/OCl^-$ by 10^6 human neutrophils stimulated by 100 ng/ml of PMA or 1 mg/ml of OpZy. Significant suppression of this function was found at 300 mmol/L of DMSO ($P < 0.01$) and 300 mmol/L of $DMSO_2$ ($P < 0.01$) for PMA-stimulated neutrophils, and at 100 mmol/L of DMSO ($P < 0.05$) and at 100 mmol/L of $DMSO_2$ ($P < 0.01$) for OpZy-stimulated neutrophils.

chrome c reduced by using the extinction coefficient of 2.1×10^4 L/mol/cm. The data were expressed as nanomoles of cytochrome c reduced after subtracting the values for the tubes containing superoxide dismutase.

Assay for production of $HOCl/OCl^-$ by stimulated neutrophils. Production of $HOCl/OCl^-$ by stimulated neutrophils was determined by a modification of the chlorination of taurine assay described by Weiss et al.[19] A standard curve was prepared with varying concentrations of NaOCl in 12 × 75 mm plastic tubes. Taurine (5 mmol/L) was added to tubes containing NaOCl or 1×10^6 neutrophils that were stimulated with either PMA (100 ng/ml) or OpZy (1 mg/ml). To this mixture, DMSO or $DMSO_2$ in varying amounts of 1, 10, 100, or 300 mmol/L was added. After a 2-hour incubation at 37° C, tubes containing cells were centrifuged, and 0.45 mmol/L TNB was added to each tube along with 100 μg/ml of catalase to eliminate any H_2O_2 that might have been present in the samples; absorbance at 412 nm was measured and compared with a standard curve of NaOCl to determine the amount of $HOCl/OCl^-$ generated. As will be seen below, the concentration of DMSO (1.4 mmol/L) added with the PMA solution was much less than that required to produce effects in this system. Also, the higher concentrations (300 mmol/L) of DMSO or $DMSO_2$ did not change the pH of the solutions.

Assay for production of H_2O_2 by stimulated neutrophils. Production of H_2O_2 was quantitated by the phenol red assay of Pick and Keisari.[36] A standard curve was prepared with varying concentrations of H_2O_2. Phenol red (0.1 mg/ml) and horseradish peroxidase (10^{-3} mg/ml) were added to tubes containing H_2O_2 or 1×10^6 neutrophils that were stimulated with either PMA (100 ng/ml) or OpZy (1 mg/ml) in the presence of 0.1 mmol/L sodium azide. To this reaction mixture either DMSO or $DMSO_2$ was added in concentrations of 1, 10, 100, or 300 mmol/L. After a 1-hour incubation at 37° C, 0.1 Eq/L sodium hydroxide was added and the absorbance was measured at 610 nm. The quantity of H_2O_2 produced by the cells was determined by using the standard curve.

Assay for production of O_2^- by stimulated neutrophils. The production of O_2^- by stimulated neutrophils was evaluated as the SOD-inhibitable reduction of cytochrome c by a modification of the method of Lehmeyer et al.[37] DMSO or $DMSO_2$ in concentrations of 1, 10, 100, or 300 mmol/L was added to tubes containing PBS, 100 μmol/L cytochrome c, and 1×10^6 neutrophils (stimulated with either PMA or OpZy, 100 ng/ml and 1.0 mg/ml, respectively). Duplicate samples were prepared containing 20 μg/ml superoxide dismutase. After a 5-minute incubation at 37° C, the tubes were centrifuged and the supernatants were read on a spectrophotometer at 550 nm. The results were converted to nanomoles of cytochrome c reduced per 10^6 neutrophils by using the extinction coefficient of 2.1×10^4 L/mol/cm. The data were expressed as nanomoles of cytochrome c reduced per 10^6 neutrophils after subtracting the values obtained for the tubes containing superoxide dismutase.

Assay for neutrophil viability. Viabilities of 1×10^6 neutrophils incubated at 37° C for 5 and 120 minutes with 1, 10, 100, and 300 mmol/L DMSO or $DMSO_2$ were determined microscopically after staining with trypan blue.

Statistics. Data were analyzed by both analysis of variance and least significant difference method if analysis of variance gave significant results. Significance was taken at $P < 0.05$. The experiments were repeated three to six times.

RESULTS

Table I summarizes the results of the cell-free experiments in which either DMSO or $DMSO_2$ was added to five products of neutrophil oxidative metabolism. The data reflect the percent reduction of the initial concentration of $HOCl/OCl^-$, H_2O_2, NH_2Cl, or TauCl, or

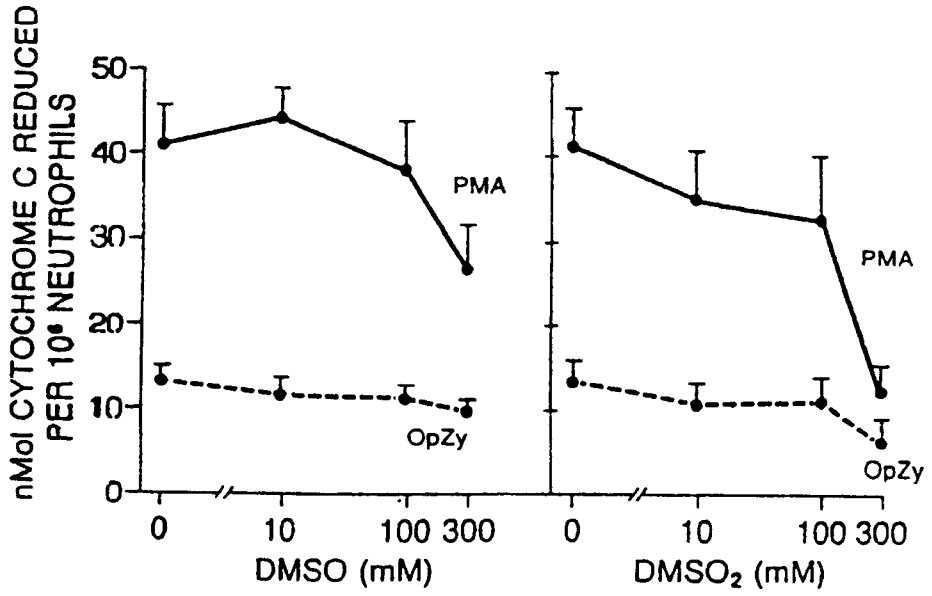

Fig. 2. Effect of DMSO and $DMSO_2$ on production of O_2^- (measured as superoxide dismutase inhibitable cytochrome c reduction) by 10^6 human neutrophils stimulated by 100 ng/ml of PMA or 1 mg/ml of OpZy. Significant suppression of this function was found at 300 mmol/L of DMSO ($P < 0.01$) and 100 mmol/L of $DMSO_2$ ($P < 0.05$) for PMA-stimulated neutrophils, and at 300 mmol/L of DMSO ($P < 0.05$) and 300 mmol/L of $DMSO_2$ ($P < 0.01$) for OpZy-stimulated neutrophils.

in the amount of O_2^- generated by the xanthine–xanthine oxidase system after a 30-minute incubation, as outlined in Methods. At concentrations as low as 1 mmol/L, DMSO, but not $DMSO_2$, was found to reduce measurable $HOCl/OCl^-$ by approximately 90%. Whereas both compounds significantly interacted with $HOCl/OCl^-$ at the higher concentrations, DMSO quenched the oxidant activity of $HOCl/OCl^-$ to a significantly greater degree at all concentrations tested. Neither compound significantly reacted with the four other substances, H_2O_2, NH_2Cl, TauCl, or O_2^-. Thus, of the five species tested, only $HOCl/OCl^-$ reacted with either DMSO or $DMSO_2$, and the major reaction was with the former.

Both DMSO and $DMSO_2$ were also evaluated for their effects on the production of oxidants by stimulated human neutrophils. At a concentration of 300 mmol/L, both compounds significantly inhibited the production of $HOCl/OCl^-$ by neutrophils stimulated by either PMA or OpZy, as shown in Fig. 1. Similarly, the production of O_2^- and H_2O_2 by PMA- or OpZy-stimulated neutrophils was also significantly inhibited by both DMSO and $DMSO_2$ at concentrations of 300 mmol/L and, in some cases, 100 mmol/L (Figs. 2 and 3). The effects of DMSO and $DMSO_2$ on neutrophil viability after a 2-hour incubation at 37° C were evaluated using trypan blue exclusion, and no significant reductions were found in three experiments (Table II).

Because of the possibility that changes in the tonicity of the medium caused by the high concentrations (300 mmol/L) of DMSO or $DMSO_2$ might have been responsible for the suppressive effects seen, similar concentrations of glycerol were also used in the $HOCl/OCl^-$ assay. In three experiments, 300 mmol/L glycerol produced only minor reductions in the amount of $HOCl/OCl^-$ produced by neutrophils stimulated by either opsonized zymosan (mean of 19.9% suppression) or PMA (mean of 15.1% suppression).

DISCUSSION

In the series of cell-free assays, neither DMSO or $DMSO_2$ in concentrations of 100 to 300 mmol/L was found to react with O_2^-, H_2O_2, NH_2Cl, or TauCl. However, DMSO, and to a much lesser extent, $DMSO_2$, did quench the oxidizing capacity of $HOCl/OCl^-$, as measured by the assay using oxidation of OPD. This result is in agreement with previous work showing that DMSO reacts with $HOCl/OCl^-$ but not with chloramines.[28]

In relatively high concentrations (100 to 300 mmol/L), both DMSO and $DMSO_2$ significantly decreased $HOCl/OCl^-$, O_2^-, and H_2O_2 production by stimulated neutrophils. In assays where NaOCl was added to a mixture of taurine and DMSO, the formation of TauCl was not inhibited, indicating that taurine reacts more rapidly with $HOCl/OCl^-$ than does DMSO (data

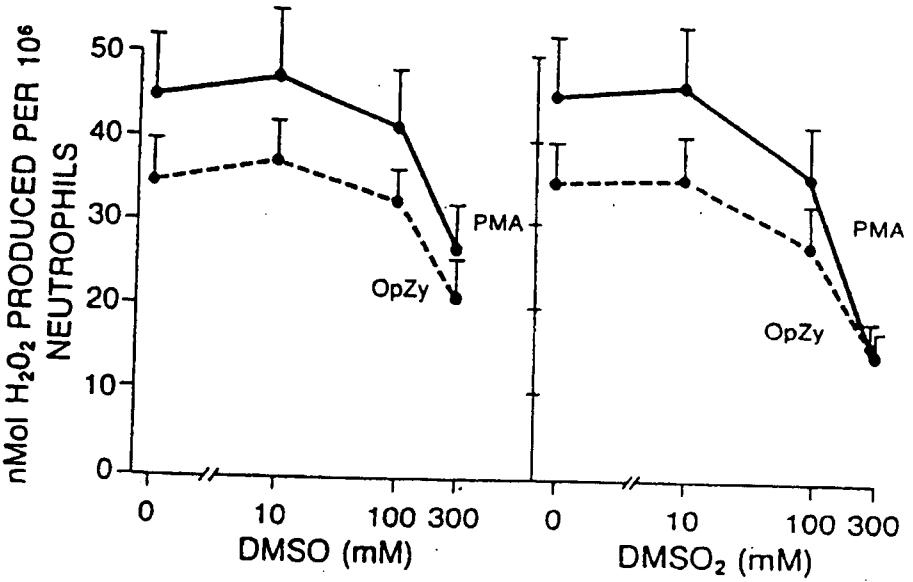

Fig. 3. Effect of DMSO and $DMSO_2$ on production of H_2O_2 by 10^6 human neutrophils stimulated by 100 ng/ml of PMA or 1 mg/ml of OpZy. Significant suppression of this function was found at 300 mmol/L of DMSO ($P < 0.01$) and 100 mmol/L of $DMSO_2$ ($P < 0.01$) for PMA-stimulated neutrophils, and at 300 mmol/L of DMSO ($P < 0.01$) and 100 mmol/L of $DMSO_2$ ($P < 0.05$) for OpZy-stimulated neutrophils.

not shown). Because neither compound was found to react directly with H_2O_2 or O_2^- in the cell-free system, it appears that DMSO and $DMSO_2$ must directly inhibit the production of these species by stimulated neutrophils rather than reacting with them and reducing their concentrations afterward. Moreover, DMSO and $DMSO_2$ inhibited $HOCl/OCl^-$ production by stimulated neutrophils to an equal degree, whereas DMSO had a much greater quenching effect on $HOCl/OCl^-$ than did $DMSO_2$ in the cell-free system.

The inhibitory effect of DMSO on the production of O_2^- and H_2O_2 suggests that this compound might have an effect directly on the membrane-associated NADPH oxidase. DMSO did not appear to actually disrupt the membranes, inasmuch as viability of the cells, at least as assessed by trypan blue exclusion, was not affected by DMSO in our study. These findings suggest the possibility that the suppressive effect of DMSO on neutrophil microbicidal activity could be caused by a mechanism other than the scavenging of hydroxyl radicals. Although DMSO might be capable of preventing the damaging effects of hydroxyl radicals produced by neutrophils, the generation of other products of neutrophil oxidative metabolism, particularly $HOCl/OCl^-$, appears to be inhibited by DMSO as well. These products have been demonstrated in a number of studies to be essential to the normal microbicidal activities of neutrophils. Furthermore, because DMSO does have the ability to quench the oxidant activity of $HOCl/OCl^-$ under the appropriate conditions, its effect as an inhibitor of tissue inflammation might also be explained in part by this effect, although DMSO may not be able to react rapidly enough with $HOCl/OCl^-$ to compete effectively for this species with other biologic compounds. Another possibility to be considered is that the DMSO preparations, $DMSO_2$ preparations, or both might contain small amounts of another, very effective inhibitor of neutrophil function.

Table II. Effect of DMSO and $DMSO_2$ on neutrophil viability

Concentration (μmol/L)	Percent viability*
Control	94.0 ± 1.2
DMSO	
1.0	92.5 ± 2.2
10.0	88.5 ± 4.1
100.0	93.5 ± 1.5
300.0	94.5 ± 1.7
$DMSO_2$	
1.0	87.5 ± 3.2
10.0	96.0 ± 0.8
100.0	84.5 ± 5.0
300.0	89.5 ± 5.4

Values are expressed as mean ± SEM of results from three experiments.
*Viability was measured as percentage of cells excluding trypan blue after a 2-hour incubation at 37° C in DMSO or $DMSO_2$.

Although the concentrations required to demonstrate significant inhibition in our assays were high (i.e., 100 to 300 mmol/L), they parallel those used by other in-

vestigators performing similar experiments. In addition, when DMSO has been used therapeutically, it has often been applied directly to the affected tissues; therefore, locally high concentrations might be reached under these circumstances. In any event, the results of the present study suggest an alternative explanation for the effects that DMSO might have as an anti-inflammatory agent.

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