CARDIAC PHARMACOLOGY OF DIMETHYL SULFOXIDE AND ITS POSTULATED RELEVANCE TO ORGAN PRESERVATION IN ISCHEMIC OR HYPOXIC STATES

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Beginning with the studies published by Sams and colleagues[1] in 1966, there has been a growing list of effects of dimethyl sulfoxide (DMSO) on the myocardium. Many of the studies of cardiac effects of DMSO focused on its use in high (multimolar) concentrations to reduce freeze-thaw-induced injury to the heart, with the ultimate goal being long-term cryopreservation of human hearts for transplantation. Studies aimed at using DMSO for cryopreservation of other organs such as the kidney were also prevalent during the 1970s. Despite the fact that these organ cryopreservation studies invariably involved organ ischemia, albeit under extremely low temperatures, little consideration was given to evaluating the effects of DMSO under more common settings of global cardiac ischemia as occurs intraoperatively during some cardiothoracic surgical procedures, or in the context of regional ischemia that can precipitate infarction. Although there were two studies of the effects of DMSO on myocardial ischemia,[2,3] the relative paucity of studies in more clinically relevant ischemic states may have been due to both an incomplete understanding of the processes contributing to ischemic injury, and a failure at the time to appreciate and apply new facts about the pharmacology of DMSO that had been elucidated in a variety of noncardiac and nonischemic settings, many of which overtly shared no obvious relationships with cardiac pathophysiology.

This paper first briefly summarizes the major pharmacological effects of DMSO on the normal mammalian myocardium, considering their possible implications to human medicine and cardiac ischemic states in particular, but also suggesting that although these effects are intrinsically interesting and demonstrate the diverse actions of this drug, they may be of little utility in currently encountered clinical situations. Evidence will then be presented to support the concept that an important component of ischemic injury to the heart and possibly to other ischemic organs is generation of oxygen radicals, including the hydroxyl radical. A proposal will then be made that the hydroxyl radical-scavenging ability of DMSO, combined with its ability to permeate cells readily, may confer upon the drug a unique and important adjunctive role for preventing this aspect of cell death in some clinical settings of ischemia.

Effects of DMSO on the Myocardium

Because of the problems associated with interpreting the direct cardiac effects of any drug with peripheral vascular effects, most of the work describing the cardiac effects of DMSO has been conducted on isolated hearts or other

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convenient *in vitro* myocardial preparations. The major actions noted below are grouped in terms of actions on cardiac contractile force development, rate and automaticity, and on myocardial structure and biochemistry. It will become apparent that most of these effects are produced by DMSO concentrations in excess of 0.1 M, concentrations exceeding those likely to occur or persist in the blood of intact animals. This singular observation is liable to restrict utilization of DMSO for its cardiac actions in clinical medicine.

DMSO can produce either positive or negative inotropy.[4] Aside from the expected dose-dependency of these responses, the nature of the response depends upon the particular muscle preparation studied (e.g., atrial versus ventricular myocardium), and upon the species of animal from which the tissue was taken. In most preparations studied DMSO concentrations of 70 mM or less increase contractile force, although the positive inotropic responses are rather transient. Above 70 mM or so the responses become particularly species-dependent. For example, guinea pig atrium develops positive inotropic responses to DMSO concentrations as high as 1.4 M, with peak positive inotropic responses occurring with DMSO concentrations around 840 mM. In contrast, negative inotropic responses predominate in feline atrium and papillary muscle and in canine trabecular muscle,[5] even with DMSO concentrations as low as 70 mM. In contrast to the transience of the positive inotropic responses to DMSO, the negative inotropic responses are usually long-lasting, even upon removal of the DMSO, and may reflect frank myocardial damage by the drug.

Osmotic effects,[6] inhibition of Na,K-ATPase,[7-9] and stimulation of adenyl cyclase activity[7] appear to play roles in the positive inotropic responses to DMSO, yet with available data it is impossible to evaluate the relative contributions of each of these possible mechanisms to overall changes of contractility. Although DMSO can release histamine,[10] which is cardiotonic in some preparations, and may also release other cardioactive mediators from mast cells or basophils, it is unlikely that these processes mediate the direct inotropic effects of DMSO seen in isolated cardiac preparations. DMSO concentrations that produce positive inotropy stimulate ATP-dependent Ca uptake by isolated striated muscle sarcoplasmic reticulum,[7,11,12] including cardiac microsomes.[7,11] Stimulation of this subcellular process is typical of some (e.g., catecholamines) but not all (e.g., digitalis glycosides) drugs that produce positive inotropy. None of the contractile effects of DMSO is antagonized specifically by beta-adrenergic blockers,[13] H_1-type antihistamines,[13] or other classical receptor blockers at doses that do not intrinsically alter cardiac contractility. Catecholamine depletion does not affect the positive inotropic responses to DMSO.[6]

In isolated rabbit atria the positive inotropic responses to isoproterenol are potentiated by low DMSO concentrations.[14] It has not been established that DMSO can potentiate the cardiotonic actions of endogenous or exogenous catecholamines or related beta-adrenergic agonists in the intact animal.

Although DMSO concentrations of approximately 140 mM or less can produce slight increases of cardiac spontaneous rate, measured in rabbit right atria not treated with other drugs, the predominant effect of DMSO at higher concentrations is a significant dose-dependent negative chronotropic effect, accompanied as noted above by significant negative inotropy. However, in contrast to the ill-defined mechanisms responsible for the direct inotropic effects of DMSO, it appears that a major mechanism for the negative chronotropic actions in these isolated preparations is inhibition of acetylcholinesterase in vagal neuroeffector junctions of the heart (e.g., nodal tissue), with suppression of intrinsic pacemaker

cell rates due to acetylcholine accumulation.[15] Evidence to support the relative specificity of this effect comes from observations that pretreating atria with atropine at concentrations as low as 1 μM can almost completely block the negative chronotropic effects of 1.4 M DMSO, an effect of DMSO that in the absence of atropine decreases spontaneous rate by almost 90%. Moreover, if one depresses spontaneous rate by initial DMSO treatment and then adds atropine while the atria are still exposed to DMSO, atrial rate will promptly return to values not far below those measured before DMSO addition. Unmasking the direct effects of DMSO on spontaneous rate by pretreating atria with atropine shows that DMSO concentrations as high as 840 mM actually produce slight, but statistically significant, increases of spontaneous rate.[15]

In the relatively simple isolated atrial preparations, DMSO decreases contractile rate without appreciably altering rhythm. The situation is different however in the intact heart, in which DMSO concentrations higher than 140 mM or so decrease rate and alter rhythm, presumably by an acetylcholine-related effect to slow conduction through the atrioventricular node. It is likely that the ability of DMSO to enhance or precipitate digitalis glycoside toxicity in the intact animal[16]—toxicity that is manifest primarily as cardiac arrhythmias—is due to synergistic effects to slow atrioventricular nodal conduction (an acetylcholine-related or vagal effect) and to inhibit Na,K-ATPase directly.

The functional effects noted above are generally reversible with short-term (1 h or less) exposure to DMSO concentrations not exceeding approximately 1.4 M, followed by DMSO removal.[17,18] Similarly, based on electron microscopic evidence, the heart undergoes no obvious ultrastructural changes with this treatment.[17,18] The absence of marked changes upon exposure to DMSO-containing solutions with such high osmolality is probably due in part to the drug's ability to permeate membranes quickly, so that osmotic gradients are dissipated quickly. Nevertheless DMSO concentrations over 1.4 M or so can produce irreversible functional and structural disruption, which at least partially may be due to extreme osmotic imbalances. When given to hearts that have been damaged by some pathological process that is likely to impart damage characterized in part by increases of membrane permeability, lower concentrations of DMSO than that noted above may provoke further damage which is not likely due to the drug's osmotic properties, but rather to some other action on membrane function and structure.

Depending upon the concentration of DMSO administered, the drug can produce a variety of metabolic stimulating and suppressing actions in various biological systems.[19] Perhaps of most relevance to myocardial bioenergetics is the potential action of DMSO on myocardial oxidative phosphorylation, which provides the majority of ATP for cardiac contraction. At very high molar concentrations, exceeding those likely to be reached *in vivo* as the result of systemic DMSO administration, the drug profoundly suppressed a variety of enzymatic indicators of mitochondrial function.[20] Lower concentrations, in the range of 10 to 100 mM, produced no apparent metabolic suppression of isolated perfused brains, and actually appeared to modestly stimulate glycolysis.[21] When added to normally respiring isolated heart mitochondria, DMSO concentrations up to 840 mM had no significant effect on mitochondrial respiratory control or on estimated rates of oxidative ATP synthesis,[22] and DMSO concentrations as high as 1.4 M only moderately slowed ADP-stimulated respiratory rates. The possible implications of these observations to the tolerance of heart and other organs to hypoxia or ischemia are discussed below.

DMSO and Oxygen Deprivation

Few if any of the cardiac effects of DMSO summarized above constitute a rational mechanism for why or how the drug might be used as an adjunct to tissue preservation in hypoxic or ischemic states, except of course for the recognized ability of DMSO to act as an effective cryoprotectant. Instead, the utility of the drug may not be due to any specific cardiac contractile or metabolic action, but may involve its ability to (1) permeate cells rapidly and extensively, reaching specific intracellular sites at which damaging metabolites may be generated; (2) aid in the cellular permeation of other known protective drugs, whether they be beta-adrenergic blockers, calcium channel blockers, antioxidants or other compounds; and, perhaps most importantly, (3) to scavenge hydroxyl radicals. This position is taken because evidence from various disciplines strongly suggests that (1) a component of "ischemic" damage to the heart involves paradoxical damage from reperfusion[23] and, specifically, from reoxygenation and the generation of cytotoxic oxygen radicals.[24,25] Similar adverse changes occur with reoxygenation of the oxygen-deprived but nonischemic myocardium. (2) Anoxia- or ischemia-induced central nervous system pathology[26,27] may involve lipid peroxidative changes and other evidence of excessive biological oxidation, some of which reflect oxygen free radical phenomena. Similar changes occur in ischemic gut[28] and they may occur in virtually all other oxygen-deprived organs that are subsequently reoxygenated. (3) In regional myocardial ischemia and infarction, local inflammatory responses involving leukocyte infiltration, thrombosis, and vascular endothelial alterations occur.[29,30] These processes may ultimately be directed at tissue repair, but they may also transiently contribute to tissue damage. Some of these processes involve free radical phenomena. Similar changes may occur during rejection of transplanted hearts.[31]

This plus other circumstantial evidence suggests that in organ ischemia mitochondrial reduction of molecular oxygen, which normally occurs by tetravalent reduction to water, may change such that there is greater formation or accumulation of univalent reduction products, including superoxide anion, hydrogen peroxide, and hydroxyl radical, the latter of which is perhaps most cytotoxic. Inflammatory processes propagated by white blood cells could also serve as an important source of oxygen radicals. Overall, hypoxia with or without ischemia can damage organs by processes including accumulation of reduced electron transport system components that may spontaneously autoxidize during oxygen deprivation;[32] accumulation of reductants during oxygen deprivation that may be innocuous in the relative absence of oxygen, but autoxidize to toxic species upon reoxygenation;[32] release of chemotactic factors from the damaged organ that, when reperfused with blood, attract and possibly activate granulocytes, producing inflammatory reactions involving oxygen free-radical generation;[32] conversion of xanthine dehydrogenase to xanthine oxidase, which in the presence of suitable substrates can generate oxygen radicals;[33] and depletion of cellular oxygen radical-metabolizing enzymes[34] and endogenous antioxidants. Any or all of the above processes may occur during organ hypoxia or ischemia, producing damaging oxygen metabolites.

If the above mechanisms do contribute to ischemic damage, then the most efficient means of dealing with them would involve enzymatically degrading superoxide anion and hydrogen peroxide so that hydroxyl radical formation from these precursors or from reaction of hydrogen peroxide with metal chelates, is reduced. Using both buffer-perfused and blood-perfused isolated heart models of

global ischemia, it has been shown[24,25] that supplementing hypothermic cardioplegia solutions with Cu-Zn superoxide dismutase and catalase significantly prevented many manifestations of ischemic damage, including loss of ventricular contractility and compliance, marked increases of coronary vascular resistance, and disrupted mitochondrial oxidative phosphorylation. While this strongly incriminates oxygen radicals as participants in ischemic damage under some conditions, many questions arise, including whether oxygen-related damage is intercepted intracellularly, extracellularly, or at both sites, and which specific oxygen metabolite(s) is (are) responsible for the damage.

Superoxide dismutase and catalase have high molecular weights (about 32,000 and 250,000, respectively), and so it is conceivable that only under extreme ischemic conditions during which cell membrane permeability has greatly increased could they gain access to the intracellular space to act upon their substrates, which are apt to be generated by the mitochondrion. With the very high molecular weight of catalase in particular, it is difficult to envisage that appreciable amounts would enter the cell unless it were so damaged as to be virtually nonviable. If the endogenous defense mechanisms for hydrogen peroxide are compromised, as suggested by the work of Guarnieri and colleagues,[34] then hydrogen peroxide could become an important source of hydroxyl radical. Since superoxide dismutase would act only to generate more hydrogen peroxide from superoxide anion, that enzyme alone could theoretically exacerbate damage by forming more precursor for hydroxyl radical. Moreover, if hydroxyl radical were a damaging species in cardiac ischemia, if it is generated largely by processes depending on mitochondrial univalent reduction of oxygen, and if the radical's reactivity is so great that it can only diffuse a few angstroms before acting upon a target molecule, then a hydroxyl radical scavenger with at least the following three properties might confer some small but discrete protection: an ability to scavenge hydroxyl radical well; an ability to reach likely sites of hydroxyl radical generation, such as the mitochondrion; and a relative lack of cellular toxicity in general, and a lack of adverse mitochondrial effects in particular. DMSO appears to fulfill these criteria.

Preliminary data[22] showed that brief preischemic perfusion of isolated hearts with 70 mM DMSO before a 2-h global ischemic period prevented significant changes of mitochondrial oxidative phosphorylation measured when the mitochondria were isolated after 1 h of cardiac reperfusion. The preservation of mitochondrial integrity was identical to that obtained when superoxide dismutase plus catalase were used as pretreatments.[24] However, unlike the results with the enzyme supplements, DMSO failed to provide added and needed preservation of myocardial contractility and coronary vascular resistance, compared to values of these parameters measured in drug-free nonischemic hearts. The lack of protection of these and other important parameters by DMSO may have been due to a failure to optimize the dose of DMSO or other aspects of the study design that affected the way in which DMSO was used. Also, persistent contractile dysfunction in the DMSO-treated hearts could reflect damage due to superoxide anion and/or hydrogen peroxide, and if this were the case one would expect that superoxide dismutase, catalase, or the two combined, but not DMSO alone, would be necessary to confer protection. Nevertheless the discrete mitochondrial effects of DMSO were consistent and significant, and were not accompanied by indications that DMSO worsened the ischemically damaged hearts.

Since the data with DMSO noted above were obtained using selected conditions, it is difficult to make conclusions about mechanisms by which the drug protected. However, it is tempting to speculate that since DMSO can

scavenge hydroxyl radical directly, and superoxide dismutase and catalase serve indirectly to suppress synthesis of hydroxyl radical precursors, that these three interventions may have protected by virtue of their effects on oxygen metabolism. Of course, the similarities of the mitochondrial protective effects of DMSO with those of the enzyme interventions may be purely coincidental, and at least in the case of DMSO the actual mechanism of protection may still be unknown and unrelated to the proposed effects on hydroxyl radical. However, of the pharmacological effects of DMSO listed so far, none of the direct cardiac effects of the drug can adequately explain its ability to provide limited, discrete, but nevertheless important, mitochondrial protection, and so the drug's hydroxyl radical scavenging ability appears to be a reasonable explanation.

Finney and colleagues[2] showed that DMSO plus hydrogen peroxide reduced ischemic damage of porcine hearts subjected to coronary artery ligation, postulating that DMSO might act by enhancing tissue delivery of oxygen derived from the peroxide. It was felt that the hydrogen peroxide might chemically produce what was tantamount to hyperbaric oxygenation, which many investigators have shown to benefit various ischemic or hypoxic states (and, of course, others have documented the damaging effects of hyperoxia in other conditions, postulating that oxygen radical-mediated cytotoxicity occurs). Finney and colleagues[2] did not measure systemic or myocardial oxygen tensions in their experiments, and this may be important since others have shown that systemic hydrogen peroxide administration produces trivial effects on blood oxygen tensions.[35] Myers and Donovan[36] showed that DMSO plus hydrogen peroxide, but not DMSO alone, increased oxygen partial pressure in devascularized (ischemic) rabbit skin flaps. Although this initially appears to support the concept that DMSO can aid in the cellular delivery of oxygen, they failed to control their experiments by evaluating hydrogen peroxide alone, so from their data it is impossible to determine whether DMSO really affects tissue oxygen tensions. Also, their data were highly species-dependent. Interestingly, Shattock and associates[37] recently showed that hydrogen peroxide concentrations greater than 30 μM are intrinsically toxic to the normal heart, and that concentrations as low as 6 μM kill hearts that are already damaged partially by ischemia. Therefore, since it is questionable whether DMSO actually enhances cellular oxygen delivery in the presence of hydrogen peroxide, and since there is a more fundamental question of whether hydrogen peroxide might damage ischemically injured tissues further, more carefully controlled experiments are needed to determine whether DMSO plus hydrogen peroxide (or hyperbaria) is a rational and effective combination for protecting or salvaging ischemic or hypoxic organs.

Leon and colleagues[3] showed that systemic administration of DMSO reduced myocardial fiber necrosis and ventricular aneurysm development in rats given high doses of isoproterenol. They postulated that DMSO might have protected either by an oxygen delivery-related mechanism, or by anti-inflammatory actions. However, one component of damage in this model of cardiac pathology is excessive stimulation of cardiac contractility and, more importantly, of oxygen demand in excess of oxygen delivery. Mitochondrial oxygen flux is also stimulated greatly. Collectively, the isoproterenol intervention induces what is essentially myocardial ischemia.[38] This excessive metabolic activity could also increase generation of cytotoxic oxygen metabolites, including hydroxyl radical, that could be scavenged by DMSO. The anti-inflammatory actions of DMSO that are also likely to involve hydroxyl radical scavenging may also be operative in the isoproterenol necrosis model.

Although there are many overt functional and morphologic similarities

between the paradoxical effects of myocardial reoxygenation and reperfusion after oxygen and flow deprivation, and those effects seen with readministration of calcium after a period of calcium depletion (the so-called oxygen paradox and the calcium paradox[39]), the underlying mechanisms of damage by oxygen and by calcium are likely to be different. Whereas there is evidence that DMSO can reduce myocardial damage due to reoxygenation,[22,40] Ruigrok and colleagues[41] have shown that 1.4 M DMSO does not protect hearts against the effects of calcium repletion after calcium depletion. However, these data[41] should be interpreted cautiously since the DMSO concentration tested was the same as the highest concentration tolerated by normal (nonischemic) myocardium[17] and so it is possible that lower concentrations may have given different and perhaps more positive results.

SUMMARY

There is a wide variety of clinical states in which organ hypoxia and ischemia followed by reoxygenation and reperfusion are unavoidable, and in which organ viability may be compromised by many factors, only one of which may involve oxygen radical-mediated damage. In organ transplantation, for example, although there are many techniques for preserving graft function (some favoring ischemic storage and others favoring continuous perfusion with nonoxygenated solutions that are hypoxic, although not truly anoxic) virtually every technique involves some duration of ischemia, oxygen deprivation, or both.[42,43] Intraoperatively, warm ischemia and reperfusion occur routinely, as in endarterectomy, renal revascularization and nephrolithotomy, streptokinase dissolution of coronary thrombi, balloon dilatation of stenotic coronary vessels, and so on. Open heart surgery relies upon myocardial ischemia, albeit facilitated by varying degrees of hypothermia and, often, cardioplegia. Organ trauma such as that to the central nervous system also involves aberrations of blood flow and oxygen delivery at normothermia, plus many other factors, such as coagulopathy (platelet aggregation, inflammation), that may be modified beneficially by one or more of the recognized properties of DMSO,[44] including its ability to scavenge hydroxyl radical. The role of DMSO in these settings is discussed elsewhere in this volume.

If DMSO is to have more widespread utility in managing organ hypoxia and ischemia, how might its diverse pharmacological properties be used most effectively? It is not likely that it can be used optimally alone as the sole drug intervention, nor at high (multimolar) concentrations, which could be intrinsically damaging. Instead it may be most useful when administered at low (less than 100 mM) concentrations, which are still biologically active, in conjunction with appropriate physical interventions such as hypothermia, and with pharmacological supplements such as membrane-stabilizing or anti-inflammatory drugs, beta-blockers, calcium antagonists, metabolic substrates, and oxygen-metabolizing enzymes, all of which are now receiving considerable attention as clinically useful cytoprotective agents. If the efficacy of these other drugs is limited in part by their relative inability to attain protective concentrations at intracellular sites, then supplemental DMSO might provide two kinds of benefits: an intrinsic ability to protect by the mechanisms noted above, plus a potential and largely untested ability to aid the cellular delivery of other protective drugs, some of which have very high molecular weights which restrict them to the vascular space or at least to the extracellular space. Evidence to support the latter synergistic effect comes

from the data of Broadwell and colleagues[45] that show dramatically that systemic administration of DMSO allows substances with molecular weights as high as 70,000 to cross the blood-brain barrier, which is exquisitely selective in its permeability characteristics. Prospective studies emanating from these observations and speculations will hopefully have a positive impact on the use of DMSO for managing organ hypoxia and ischemia.

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