Dimethyl Sulfoxide (DMSO) and Glycerol, Hydroxyl Radical Scavengers, Impair Platelet Aggregation Within and Eliminate the Accompanying Vasodilation of, Injured Mouse Pial Arterioles*

WILLIAM I. ROSENBLUM, M.D., AND FAROUK EL-SABBAN, PH.D.

SUMMARY The hydroxyl radical scavengers dimethyl sulfoxide (DMSO) and glycerol were effective inhibitors of platelet aggregation in an in vivo mouse model of pial arteriolar injury. Aggregability was expressed in terms of the time required for a noxious stimulus (light + dye) to initiate aggregation. These drugs, given 1 hour before the injury, also eliminated the dilation which accompanied the damage. The same drugs failed to influence the constriction which accompanied an identical injury to mouse mesenteric arterioles, but again impaired platelet aggregation in the damaged mesenteric vessel. The data support the concept recently introduced by others, that, in the brain, hydroxyl radicals may mediate vascular damage and/or dilation accompanying the damage. The data also support the concept that platelet aggregation may be stimulated, directly or indirectly, by hydroxyl radicals. The effects of DMSO and glycerol in this study, irrespective of the molecular basis for the effects, may be relevant to the reported therapeutic benefit of these agents in cerebrovascular disease.

THE reported effects of dimethyl sulfoxide (DMSO) are protean and include the ability to impair platelet aggregation1,2 and to reduce brain damage after occlusion of cerebral blood vessels perhaps by reducing cerebral edema.3,4 The mechanisms by which DMSO exerts its actions are unknown, but might include its capacity to scavenge hydroxyl radicals,5,6 a free radical species with potentially damaging effects. Since there are almost no studies of DMSO's action on platelet aggregation in vivo,1 particularly in cerebral blood vessels, and in view of reports concerning its beneficial action in cerebrovascular occlusion, it seemed essential to perform additional studies of DMSO's effects on the cerebral vasculature. This seemed particularly important in the light of renewed interest in the pathologic effects of free radicals6 and the failure of earlier workers to consider the free radical scavenging ability of DMSO when explaining their results. The following study not only assays the ability of DMSO to impair platelet aggregation in injured cerebral microvessels, but also investigates the effects of DMSO on the alterations in vascular tone which accompany platelet aggregation in this particular model of microvascular injury. The action of DMSO in cerebral microvessels was compared with its action on mesenteric microvessels in the same model of injury, and with the action of glycerol, another scavenger of the hydroxyl radical,7,8 that has also been reported to be of value in treating occlusive cerebrovascular disease.7,8

Methods Male ICR mice were anesthetized with urethan and either the surface vessels of the brain (pial vessels) or the mesenteric vessels exposed as previously reported.9,10 The method of injuring the vessels and inducing platelet aggregation has been extensively described in earlier publications.9-11 Briefly stated, the vessels were observed with a Leitz Ultropak microscope that employs epi-illumination with either a tungsten or a filtered 200 W mercury lamp. The filters include a Leitz BG-12 exciter filter as well as heat and UV filters. The present studies employed a 10 x ocular and 22 x objective with an immersion attachment. With the mercury lamp the intensity of illumination at the focal plane with all filters in place was 23 X 10^6 W/cm^2 when measured daily with a silicon diode detector and radiometric filter. The filtered light from
the mercury lamp produced no evidence of microvascular damage unless sodium fluorescein was present in the circulation. The latter, like the light, is non-toxic by itself. But when 2% sodium fluorescein, 0.2 ml/25 grams body weight, was injected via the tail vein, vascular damage and platelet aggregation rapidly occurred following illumination with the mercury source. The aggregates fluoresced and were readily visible as they adhered to the damaged endothelium. Prior to injecting the fluorescein, observation was performed with the tungsten lamp. Immediately upon injection of fluorescein, which took a second, the illumination was switched to the mercury lamp and this was continued throughout the period of observation. The time required for aggregation to be initiated was measured with a stop watch started the instant the illumination was switched to the mercury source and stopped the instant the first adhering aggregate was recognized. During the brief period of observation, a drop of artificial cerebrospinal fluid (brain) or saline (mesentery) was held between the tip of the immersion attachment and the tissue.

Arterioles are the subject of this presentation. Their internal diameter was measured with an ocular micrometer before induction of aggregation and again after aggregation was complete and the vessel occluded by platelet thrombi. The fluorescent aggregates made it easier to determine the internal dimensions of the vessels by often providing a clearer boundary between contents and wall than that provided by tungsten illumination. In some of the studies, the 2-slit technique of measuring red cell velocity was employed with the aid of a TV microscope, 2-slit velocimeter, and cross correlator (IPM, San Diego CA). This gave RBC velocity at the center of the vessel and from this the shear rate at the wall could be calculated. These measurements were taken in the minutes preceding induction of aggregation, using the mercury light for approximately one minute with a green filter replacing the BG-12 filter. This enabled us to determine whether shear rates were systematically altered by the DMSO or glycerol given one hour earlier. We were concerned about this because preliminary studies showed a modest but definite relationship between shear rate and aggregation latency. The DMSO and glycerol were injected one hour before inducing aggregation. The highest dose was 100% DMSO or glycerol .05 ml/25 g body weight. Lower doses were made by diluting the drugs with saline, and injecting the same volume (.05 ml/25 g).

TABLE 1  Effect of DMSO on Platelet Aggregation and Change of Diameter in Injured Pial Arterioles

<table>
<thead>
<tr>
<th>Dose DMSO (ml/kg body weight)</th>
<th>5.0 (N = 10)</th>
<th>0.5 (N = 10)</th>
<th>0.05 (N = 10)</th>
<th>Saline (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seconds to initiate aggregation</td>
<td>117 ± 49*</td>
<td>71 ± 25</td>
<td>51 ± 13</td>
<td>57 ± 20</td>
</tr>
<tr>
<td>Initial diameter (μm)</td>
<td>39 ± 5</td>
<td>39 ± 4</td>
<td>37 ± 8</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>Diameter after aggregation (% control)</td>
<td>95 ± 18*</td>
<td>112 ± 11</td>
<td>124 ± 15</td>
<td>122 ± 19</td>
</tr>
</tbody>
</table>

*ANOVA p ≤ 0.001.

DMSO significantly prolonged the time required for the noxious stimulus to initiate platelet aggregation in damaged pial arterioles (p < 0.001, analysis of variance). The accompanying dilation was also significantly impaired, and at the highest dose of DMSO the dilation was eliminated, with many damaged vessels constricting instead (p < 0.001, analysis of variance).
Table 2: Effect of Glycerol on Platelet Aggregation and Change of Diameter in Injured Pial Arterioles

<table>
<thead>
<tr>
<th>Dose glycerol (ml/kg body weight)</th>
<th>Initial diameter (μm)</th>
<th>Diameter after aggregation (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 (N = 10)</td>
<td>36 ± 7</td>
<td>98 ± 23†</td>
</tr>
<tr>
<td>2.5 (N = 10)</td>
<td>36 ± 5</td>
<td>111 ± 20</td>
</tr>
<tr>
<td>0.25 (N = 10)</td>
<td>34 ± 6</td>
<td>118 ± 30</td>
</tr>
<tr>
<td>Saline (N = 10)</td>
<td>35 ± 5</td>
<td>133 ± 19</td>
</tr>
</tbody>
</table>

*ANOVA p < 0.001, †ANOVA p < 0.02.
Glycerol significantly inhibited platelet aggregation and eliminated dilation in injured pial arterioles, (analysis of variance p < 0.02 for the first effect and < 0.001 for the second).

Discussion

The data clearly show a dose dependent lengthening effect of DMSO and of glycerol on the latency of platelet aggregation in injured pial arterioles, and a dose dependent reduction in the dilation that accompanies injury and aggregation. Since both DMSO and glycerol scavenge hydroxyl radicals, the similarity of the two drugs' actions in our model of cerebrovascular injury supports the hypothesis that hydroxyl radicals may be important mediators of the cerebral microvascular responses in the model. The similarity of glycerol's effect to that of DMSO would then also support the suggestion that hydroxyl radical scavenging accounts for many of DMSO's pharmacologic effects.

It has been proposed that the injury in our model is produced by heat generated as the dye absorbs light, in the manner of a laser whose energy is absorbed by carbon particles or Evan's blue. It is possible that radicals are produced as a result of heat induced damage. However it is also possible that the injury is produced by radicals generated at the time the fluorescein is excited. There is precedent for radical formation as a cause of cerebrovascular injury with resultant dilation of pial arterioles. In either case, that is whether a cause of injury or a result of injury, the hydroxyl radical could cause dilation, and the latter could then be inhibited by radical scavengers.

Platelet aggregation in our model is thought to be stimulated by the microvascular damage, since it does not occur outside the illuminated field. Damaged endothelium is observed with electron microscopy. However, aggregation latency is increased by drugs that impair aggregation in vitro but not by an anti-inflammatory drug with little effect on aggregation in vitro. This suggests that the antiaggregating action of drugs in our model can be the result of a direct action of the drugs on the platelets, rather than the consequence of a protective effect on the vessel wall. We cannot rule out the latter in the present study, because we have not compared the morphologic changes in the vessel wall of control animals with those seen in the DMSO or a glycerol treated group. In considering the alternative, namely an effect of DMSO and glycerol on platelets themselves, it is pertinent to point out that DMSO has been found to impair aggregation of human platelets in vitro. In vitro studies of DMSO's action on mouse platelets have not been reported. If DMSO inhibits aggregation through an action on the platelet, the question would remain as to whether this effect is mediated by hydroxyl scavenging within the platelet. Since the radical is thought to be destructive, it would be difficult to imagine a facilitatory role for the radical in platelet aggregation. However small amounts of some reactive species are thought essential to the activity of cyclooxygenase, an enzyme implicated in platelet aggregation.

Dilation and aggregation may appear related in this

Table 3: Effects of DMSO on Platelet Aggregation and Change of Diameter in Injured Mesenteric Arterioles

<table>
<thead>
<tr>
<th>Dose DMSO (ml/kg body weight)</th>
<th>Initial diameter (μm)</th>
<th>Diameter after aggregation (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 (N = 10)</td>
<td>72 ± 37*</td>
<td>49 ± 27†</td>
</tr>
<tr>
<td>0.5 (N = 10)</td>
<td>50 ± 30</td>
<td>49 ± 24†</td>
</tr>
<tr>
<td>0.05 (N = 10)</td>
<td>68 ± 18</td>
<td>49 ± 24†</td>
</tr>
<tr>
<td>Saline (N = 10)</td>
<td>68 ± 15</td>
<td>49 ± 20†</td>
</tr>
</tbody>
</table>

*ANOVA p = 0.2, ANCOVA with shear rate as covariant p = 0.1. If saline, 0.05 and 0.5 ml/kg are pooled and compared with 5 ml/kg, the effect of the latter is significant at the 0.05 level. Only the highest dose of DMSO inhibited platelet aggregation in injured mesenteric arterioles. Analysis of variance only shows p < 0.1, even when means adjusted to take differences in shear rate into account (see text). There was no effect of DMSO on the constriction accompanying damage and aggregation in this vascular bed.
study since the former was reduced as the latter was impaired. As they aggregate, platelets release materials, some of which may produce dilation and others constriction.\textsuperscript{22} We cannot rule out the possibility that DMSO and glycerol eliminated dilation by eliminating the release or production of a dilator by platelets. However, of all the vasoactive substances released by platelets, thromboxane seems most potent and this is a constrictor.\textsuperscript{23}

The effect of DMSO and glycerol on platelet aggregation in mesentery was similar to that seen in brain, except that the inhibitory effect of DMSO was not statistically significant with the sample size used, unless we combined the 2 lower dose groups with the saline control, and compared this combined group with the group receiving the highest dose of DMSO. Using the same model of microvascular injury, we have previously shown that drugs which impair aggregation in vitro, and in cerebral microvessels, can actually accelerate aggregation in mesenteric vessels.\textsuperscript{10} This difference in drug actions on brain and mesenteric vessels presumably related to a difference in substance(s) produced by the two tissues or to a differential effect of drugs on these tissues.\textsuperscript{30} Alternatively, at least in the present study, we should consider the possibility that the weaker effect of DMSO on platelet aggregation within mesenteric vessels may be related to the route of drug administration. In the studies of cerebral vessels intraperitoneal injection was used, while a subcutaneous route was employed in the studies of mesentery. If lower blood levels were achieved by the latter route, and this accounts for the weaker effect of DMSO, the same phenomenon must not have applied to the study of glycerol, where a powerful antiaggregatory effect was seen in both brain and mesentery, though once again a subcutaneous route was employed for studies of the latter.

A further difference between the cerebral and mesenteric vessels is reflected in the fact that the mesenteric arterioles constricted while the pial arterioles dilated.\textsuperscript{28} We have shown that cyclooxygenase inhibitors, which impair platelet aggregation in the brain, increase the dilation of the pial arterioles and reduce the constriction of the mesenteric arterioles.\textsuperscript{28} The latter results suggest that prostaglandin synthesis may result in production of a vasoconstrictor(s) whose action in the pial arterioles is overcome by the presence of a dilator, produced by a pathway independent of cyclooxygenase. As suggested above, abolition of dilation by hydroxyl radical scavengers permits the hypothesis that the dilator in question was a hydroxyl radical. Moreover, at the highest doses of DMSO and glycerol an absolute constriction, rather than dilation, was observed for many arterioles. However, the data do not suggest an explanation for the failure of the putative dilator to dominate the response of mesenteric arterioles. Nor can the data explain why constriction of mesenteric arterioles failed to increase if a dilator was eliminated by the scavengers.

The disparities between the results in the brain and those in the mesentery do not rule out the hypotheses that hydroxyl radicals mediate microvascular injury in our model and play a role, directly or indirectly, in stimulating platelet aggregation. The disparities emphasize the need for caution, both with respect to the use of so simplistic an explanation as hydroxyl radical scavenging in interpreting the results from brain vessels, and also with respect to generalizing results from one vascular bed to another. Nevertheless, questions concerning mechanisms of response and generalization of conclusions should not obscure the fact that two hydroxyl radical scavengers did significantly alter the responses to microvascular injury in the brain with respect to platelet aggregation and vasodilation. Our data may be related to reports of a beneficial effect of DMSO and glycerol in cerebral infarction.\textsuperscript{5,4,7,8} The success or failure\textsuperscript{28} of these therapies could conceivably hinge on the importance of platelet aggregation or vasodilation in exacerbating the effects of the original vascular occlusion.

\textbf{References}

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Balloon Catheter as a Model of Cerebral Emboli in Humans

GY. GÁCS, M.D., F. T. MÉREI, M.D. AND M. BODOSI, M.D.

SUMMARY A striking similarity has been found between the distribution of occlusions in various cerebral arteries and the statistical regularity of the pathways of balloon catheters drifting freely in the blood stream. Based on the assumption that the course of a balloon is determined by the same hydrodynamic laws as that of emboli of similar size, it is concluded that the majority of occlusions of the cerebral arteries are of embolic origin. Emboli, then, might also be an explanation for most of the TIA's.

Knowledge of the pathogenesis of occlusions of the intracranial arteries would be important to therapy. Despite several studies of this problem it cannot be decided whether a particular occlusion has its origin in a local thrombus or in an embolus. Recently the theory of the embolic origin of intracranial vascular occlusions has been favored. However, primary evidence for it is still lacking.

There are considerable, regular differences in the incidence of occlusions among the individual intracranial arteries. These differences are regarded by several workers as a proof of local thromboses because emboli might be expected to have an irregular distribution. Others consider possible a certain regularity in the distribution of occlusions even in the case of emboli, because of the laminar blood flow in the cerebral arteries. Our balloon catheterizations seem to be suitable for checking the probable course of emboli in the main human intracranial arteries.

Materials and Methods

A total of 960 angiographies performed in patients having had ischemic events in the carotid territory were analyzed in order to determine the frequency of occlusions of intracranial arteries. One hundred and forty-two of the 960 patients had TIA's, while the others had strokes of varying severity. In 221 of the 960 angiograms we found intracranial 

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