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Failure to prevent decompression illness in rats by pretreatment with a soluble complement receptor

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Broome JR, Pearson RR, Dutka AJ. Failure to prevent decompression illness in rats by pretreatment with a soluble complement receptor. Undersea Hyperbaric Med 1994; 21(3):287–295.—Controversy exists over the role of complement activation in the natural history of decompression illness (DCI), and whether an individual's predisposition to DCI might be influenced by susceptibility to activation of complement by intravascular gas bubbles. Treatment with a soluble complement receptor (sCR-1), which neutralizes activated complement components, is known to be beneficial in other complement-dependent disease processes. This study investigated the effect of treating rats with sCR-1 or saline before decompression from a dive profile known to produce a high incidence of DCI. No statistical difference in the incidence of DCI was observed between the 27 rats treated with sCR-1 and 26 control rats treated with saline. The study was unable to confirm the previously reported observation in rats of a positive correlation between DCI incidence and increasing weight.

decompression illness, decompression sickness, diving, complement, soluble complement receptor, sCR-1, rats, weight

Many features of the etiology, progression, and subsequent pathology of decompression illness (DCI) remain to be understood and a controversial issue is the part played by the activation of the complement cascade by bubbles of inert gas generated by decompression.

In rabbits, Ward and colleagues (1) reported that individual susceptibility to activation of complement, measured in vitro after subjecting a blood sample to inert gas bubbling, correlates strongly with the risk of DCI. The in vitro activation of human complement by nitrogen bubbles has been demonstrated (2), and a further study by Ward et al. (3) in divers suggested that their observations on susceptibility to DCI in rabbits might also apply to humans.

The rabbit studies utilized an indirect assay technique which relies on the ability of activated complement to aggregate leukocytes (4). The direct assay techniques available for human complement activation products are not easily comparable with the rabbit assay. Zhang and colleagues (5) were unable to confirm in human divers

the findings in rabbits regarding sensitivity to complement activation. Other, more recent, reports further confuse the picture (6–9).

A study carried out at this institute (10) found that divers who suffered from DCI had greater levels of activated complement than asymptomatic divers, but a diver suffering from acute sinusitis also had raised levels, implying that the finding of elevated levels of complement activation products is not a specific diagnostic test for DCI.

In our study we adopted a different approach to the problem by investigating whether blockage of the complement activation cascade could influence the incidence and severity of DCI in rats. An experimental soluble human recombinant complement receptor (sCR-1), developed by SmithKline Beecham, has been shown to block C3 and C5 convertase activity and to inhibit both human and rat complement-mediated hemolysis (11). It has also been demonstrated to reduce myocardial damage following experimental ischemia (11), protect against graft rejection in an experimental model (12), and protect against complement and neutrophil-mediated lung and dermal vascular injury in vivo (13). Thus, sCR-1 would seem capable of modifying or protecting against a wide range of conditions involving complement-dependent tissue damage. Several studies have demonstrated sCR-1 to be effective in cumulative doses of 15–25 mg/kg and no adverse effects have been reported in this dose range (13).

We hypothesized that if the sequelae of complement activation played a significant role in the production of DCI in rats, then blocking the complement cascade by sCR-1 would reduce the observed incidence of DCI in rats exposed to a dive profile known to produce DCI in about 50% of animals dived.

MATERIALS AND METHODS

Prior ethical approval for the study was obtained from the Naval Medical Research Institute Animal Care and Use Committee.

Subjects were male Sprague–Dawley rats in the weight range 300–400 g. Before surgery, pairs of rats of similar weight were selected and each rat was familiarized with the Rota-Rod (Columbus Instruments), a standard rodent locomotion testing apparatus consisting of a rotating drum which can accelerate from 0 to 80 rpm in 2 min. This device trains the rat to make maximal effort to remain walking on the revolving drum by use of a short-lived, low-voltage electrical stimulus in the Rota-Rod floor. While the rat stays on the drum it remains insulated from the stimulus.

Training "runs" on the Rota-Rod were conducted over a 32-min period, consisting of a series of nine runs at 4-min intervals. For each run in a series, the length of time the rat remained on the Rota-Rod drum was automatically recorded. All rats completed one series of nine runs immediately before surgery.

Surgical methods

Each pair of rats received surgery simultaneously. Premedication with xylazine 5.0 mg/kg (Rompun, Mowbay Corp) was administered i.m., followed 15–20 min later by ketamine 30 mg/kg (Ketaset, Fort Dodge Laboratories) i.m. to achieve full surgical anesthesia.

Using strict aseptic technique, 1% xylocaine (Astra) was injected subcutaneously into the neck to achieve additional local anesthesia. A ventral incision was then made in the neck, and the tissues were dissected to expose the right internal jugular vein. A sterile PE-50 polyethylene catheter (Intramedic, Clay Adams) was then inserted into the vein toward the heart. The catheter was secured in the vein, the cranial part of the vessel tied off, and the subcutaneous tissues sutured closed. More xylocaine was then injected subcutaneously into the dorsal aspect of the neck, and a second small incision made. A subcutaneous tunnel was formed to connect the ventral and dorsal incisions, and the free end of the polyethylene catheter was passed through the tunnel to exit via the dorsal incision. Finally, both skin incisions were sutured closed.

After surgery, the rats received chloramphenicol 200 mg/kg (Chloromycetin, Parke Davis), i.v., through the catheter to reduce the risk of experimental failure due to postoperative infection. To maintain its patency the catheter was filled with a solution containing 2 U/ml of heparinized saline. It was then sealed with a knot, coiled, and secured under a fitted jacket (Alice King) designed to allow locomotion while preventing the rat from disturbing or gnawing through the catheter. We found that taping the jacket in place assured additional security.

Postsurgical/predive Rota-Rod training

In the 48 h between surgery and the dive, rats were kept in separate cages and were allowed to feed normally. Most rats initially lost weight during this period. They received no additional medication, but the indwelling catheter was flushed daily with heparinized saline to preserve its patency. Twenty-four and 30 h after surgery, rats were weighed and underwent repeat training runs on the Rota-Rod. Then, at 48 h after surgery (immediately before diving) the rats were again weighed and ran a final predive series on the Rota-Rod. These run times and weights were recorded as the predive baselines.

Dive period

In preparation for the dive, the polyethylene catheter from each rat was threaded through the lumen of a thin, tubular steel spring which was then attached to the back of the jacket on each rat. Both rats were then placed in their respective cages and the free end of each spring (and catheter) was passed through a hole in the roof of each cage and secured. This arrangement prevented the rats from gnawing through the catheters during the dive. The two cages were then placed together in the compression chamber and, taking great care to eliminate any air bubbles from the tubing, the catheters were joined to corresponding polyethylene tubing fixed to penetrations through the pressure wall of the chamber. This system allowed the rats to be injected intravenously with sCR-1 or placebo (saline) while still in the chamber under pressure. Rats were then compressed, breathing air, to 175 feet of seawater (fsw) (536 kPa) for 2 h. The chamber was flushed every 30 min to prevent build up of carbon dioxide. Rates of both compression and decompression were linear at 60 fsw/min (184 kPa/min).

Ten minutes before commencement of decompression, the control rat was injected with a bolus of 2 ml normal saline, while the other animal received 2 ml of sCR-1 (2.78 mg/ml resulting in a dose of 15–20 mg/kg depending on the weight of the rat).

This bolus was followed every 2 min by injection of 0.1 ml of saline or sCR-1 (0.5 mg/ml resulting in a dose rate equivalent to infusing 4–5 $mg \cdot kg^{-1} \cdot h^{-1}$). The every-2-min injections were continued (totaling seven in all) until the rats had surfaced from the dive and were removed from their cages. Injections rather than pump infusion were performed because no pump was available capable of the accurate delivery of the small volumes required over the range of pressure changes experienced.

In an attempt to avoid potential bias, both the chamber and Rota-Rod operators were unaware which animal received the active treatment.

Postdive period

During removal of the rats from the chamber, catheter lines were inspected for evidence of any air leakage into the system which would have caused venous air embolism on injection through the line (animals where this had occurred were invariably dead or dying on surfacing).

After surfacing, a standardized period of 5 min was allowed to elapse before the rats commenced postdive Rota-Rod runs. This permitted adequate time to open the chamber, disconnect the catheter lines, and transfer the animals to the Rota-Rod. The Rota-Rod testing schedule was at the same 4-min intervals over a period of 32 min as used for the predive training and control tests. Inability to continue the Rota-Rod evaluation or death were taken as evidence of DCI. Any animal not succumbing to DCI by the end of the Rota-Rod run was euthanized by an intravenous dose of pentobarbital (80 mg/kg) administered via the jugular catheter.

RESULTS

Seventy-two rats underwent the surgical procedure described. Of these, 12 rats either died subsequent to the surgery or managed to pull out or gnaw through their catheter.The remaining 60 rats were all dived. Seven of these were excluded from the subsequent analysis due to air leakages into the catheter lines or due to displacement, blockage, or twisting of the catheter during the dive, which prevented injection of sCR-1 or saline.

Of the 53 rats included in the analysis, 27 had received sCR-1 and 26 were controls (1 rat, included as a control, had completed Rota-Rod testing after injection of only 0.5 ml normal saline before the catheter clogged).

Overall, 34 (64%) rats lived and 19 (36%) died or were unable to complete Rota-Rod testing due to DCI. Of the 27 rats that received sCR-1, 19 lived and 8 (30%) died or exhibited signs of DCI. Of the 26 control rats, 15 lived and 11 (42%) suffered DCI (Fig. 1). The difference between the sCR-1 and placebo treatment groups is not statistically significant ($\chi^2 = 0.92$; $0.5 > P > 0.1$).

Thirty-four rats (64%) completed postdive Rota-Rod training (Table 1). For each rat, the mean of the nine Rota-Rod runs comprising the predive Rota-Rod session was subtracted from the mean of the nine Rota-Rod runs from the postdive session. A positive value would indicate that the mean performance had improved, whereas a negative value would indicate a deterioration in performance. Examination of the means of pre- and postdive Rota-Rod times showed a slight improvement for survivors in both sCR-1 and control groups (+3.11 s, SD 4.80 for sCR-1, and +3.54 s, SD 8.76

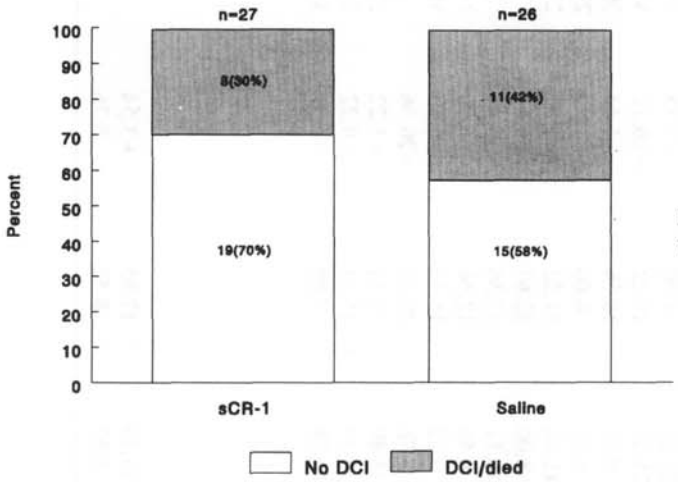

FIG. 1—Fate of rats pretreated with sCR-1 vs. saline.

for saline) but this improvement was only statistically significant for the sCR-1 group ($t = 2.82$; $P < 0.02$). Comparison between the postdive mean Rota-Rod times of the sCR-1 and saline groups suggested that the mean improvement was significantly greater for the control group ($t = 2.708$; $P < 0.01$). However, as a normal distribution cannot be assumed, the values were also ranked and statistical comparison performed by Wilcoxon's Rank Sum test for unpaired samples. The difference between the sCR-1 and saline groups failed to approach significance at the 5% level ($n_1 = 15$, $T_1 = 268.5$; $n_2 = 19$, $T_2 = 256.5$).

The weights of rats on the day they were dived ranged from 271 to 352 g, but the mean weights of the rats (irrespective of treatment) suffering DCI (310.21 g, SD 20.02) was not significantly different from those unaffected (306.51 g, SD 19.59) ($t = 0.6533$; $P > 0.5$).

DISCUSSION

Generally it is believed that if a pharmacologic preparation such as sCR-1 is to be clinically useful in human DCI, reduction in DCI mortality or incidence to around zero would be desirable in animal studies.

In our rat study, despite pretreatment with sCR-1, mortality from DCI remained high and indistinguishable from the control group. The study had sufficient power to detect a real fall in DCI incidence (had it occurred) from the 42% observed in the control group to 10% in the sCR-1 treated group, with a probability of 0.8 (14). However, several confounding factors could have influenced the experimental findings.

From previously reported studies (11–13), we expected our selected dose of sCR-1 to be optimal, but to confirm this we had intended to take blood samples via a carotid artery catheter in the immediate postdive period. Unfortunately, in a pilot study, the postoperative survival in rats with both carotid and jugular catheters was only 50%, and because of this the carotid catheter insertion was abandoned. Timely blood draws using the jugular catheter proved impossible and we were thus denied

Table 1: Rota-Rod Run Data for SCR-1 and Saline Groups of Rats

	SCR-1					Saline			
	Means of 9 Rota-Rod Runs, seconds					Means of 9 Rota-Rod Runs, seconds			
Rat No.	Predive	Postdive	Change	Rank	Rat No.	Predive	Postdive	Change	Rank
14	6.89	8.56	+1.67	19	17	9.78	12.67	+2.89	16
16	21.11	21.56	+0.45	23	18	11.22	21.33	+10.11	5
31	17.78	12.56	−5.22	32	29	23.22	15.90	−7.33	33
37	13.11	9.00	−4.11	30	32	25.33	15.22	−10.11	34
39	18.67	25.44	+6.78	9	36	9.11	10.56	+1.44	20.5
41	14.11	22.67	+8.56	8	43	9.89	8.00	−1.89	29
46	10.00	12.67	+2.67	17	45	11.78	11.22	−0.56	25
48	15.00	24.67	+9.67	7	47	8.00	22.67	+14.67	3
49	11.67	14.78	+3.11	15	50	16.11	32.56	+16.44	2
54	6.89	10.78	+3.89	14	52	8.56	12.56	+4.00	13
59	24.89	24.00	−0.89	27	55	9.22	15.56	+6.33	10
60	6.22	18.78	+12.56	4	57	8.78	29.33	+20.56	1
61	6.67	16.56	+9.89	6	67	33.89	35.11	+1.22	22
63	12.00	13.44	+1.44	20.5	70	15.33	15.11	+0.22	24
72	18.89	18.22	−0.67	26	71	14.78	9.89	−4.89	31
74	21.11	23.00	+1.89	18	$n = 15$				
76	9.56	7.89	−1.67	28					
77	9.78	14.00	+4.22	12					
81	14.67	19.56	+4.89	11					
$n = 19$									
Means	13.63	16.74	+3.11			14.33	17.85	+3.54	
SD	5.57	5.77	4.80			7.57	8.52	8.76	

our facility for quick and easy blood sampling postdive. We do not suspect that deterioration of sCR-1 activity occurred while in frozen storage before administration, because a sample we returned to the suppliers for assay toward the end of the study showed full activity. Consequently, we have no reason to doubt that the sCR-1-treated rats received sufficient active drug, but we were regrettably unable to measure the extent of complement system blockade directly, so the effectiveness of the dose given was not unequivocally confirmed.

Similarly, we were unable to confirm an effect of SCR-1 on the C3a and C5a (anaphylatoxin) components of complement activation, which Ward et al. (3) believed had a major role in DCI. Although sCR-1 is known to block both the classical and alternative pathways of complement activation, confirmation of its effect on the anaphylatoxins would have been reassuring.

The small dose of heparin administered to maintain catheter patency could have affected coagulation factors or platelet function. If complement activation in DCI is related to these heparin-influenced factors, then an effect of sCR-1 could conceivably be masked.

It also is conceivable that the predive surgery and the presence of an indwelling jugular catheter caused marked complement depletion in all rats before the dive, thus causing complement levels to be too low to influence the outcome (whether activation products were blocked or not). Against this, we argue that if complement depletion had indeed occurred, and complement was an important determinant of outcome in our rat model, then a notable reduction in DCI incidence (particularly mortality) would be expected. This was not observed, and the DCI incidence in our rats was little changed from that reported by Lillo et al. (15), on which our choice of dive profile was based.

We found no significant change in mortality rate or incidence of severe DCI between the sCR-1-treated and control groups, and in this context the postdive Rota-Rod results, intended to detect subtle differences due to mild DCI, take on less importance. No mild DCI was convincingly detected, but it is notable that for both groups of surviving rats the mean Rota-Rod performance actually improved slightly after the dive. It seems most likely that this improvement was due to a continued training effect of the Rota-Rod schedule. When analyzed by a parametric test, the mean improvement was significantly worse for the sCR-1 group than for the controls (3.11 vs. 3.54 s, $t = 2.708$; $P < 0.01$), but using the non-parametric Wilcoxon's Rank Sum test the difference between the two groups was not significant. Given the mortality results, a detrimental effect of sCR-1 on outcome that is confined to mild DCI seems unlikely. Additionally, if a training effect existed, analysis of these data by t test is inappropriate because a normal distribution of the data cannot be assumed. Probably the training effect due to the Rota-Rod schedule confounds meaningful interpretation of the postdive Rota-Rod data from the surviving rats; however, the rats that developed DCI invariably had severe signs or died, and a training effect would not influence these outcomes.

In our study the lack of any observed relationship between increasing rat body weight and DCI incidence is surprising because a strong positive association was reported by Lillo and colleagues (15) in rats within a somewhat lower weight range. We found no evidence of this association in rats we dived (weight range 271–352 g), although our study had sufficient power to detect a 6-g difference between the mean weights of affected and unaffected groups with 95% confidence.

It may be that weight loss specifically associated with surgery makes rats more susceptible to DCI and this masked our detection of a weight effect. However, reduction in muscle and adipose tissue bulk by post-surgical catabolism would tend to reduce the overall gas burden while pulmonary gas elimination remains unchanged, and this might be expected to accentuate rather than mask any decrease in DCI rate due to weight loss. In our study, the mean post-surgical weight change of rats suffering DCI (−28.76 g, SD 9.53) was virtually identical ($P > 0.5$) to the mean weight change in the unaffected group (−28.45 g, SD 10.47). In the absence of any other published data on the effect of surgery and DCI risk we can offer no convincing explanation other than to comment that in studies by Lillo et al. (15) rats were decompressed almost explosively over 10 s, whereas our rate of decompression was 60 fsw/min.

To conclude, we were unable to show that pretreatment of rats with sCR-1 had any significant effect on DCI incidence when compared to a control group. However, the implications of experimental results obtained from rodent studies of DCI must be interpreted with caution because DCI in rodents tends to manifest as an "all or nothing" phenomenon (16), contrasting with the spectrum of milder, usually sublethal DCI observed in humans or larger animals. Nevertheless, although our findings do not exclude the possibility of a role for complement in human DCI, we believe they strongly suggest that complement components, which are amenable to blockade by SCR-1, play neither an exclusive nor a major role in the genesis of DCI in rats.

We also conclude that our study highlights some of the shortcomings of rodents as a model for human DCI. A larger animal model of sublethal DCI, more akin to that seen in humans, might be preferable if further research on the influence of complement manipulation on DCI incidence is undertaken.

Despite the above comments, the tentative implication of our findings for human diving is that, whatever the role of complement, it may be unrealistic to expect that pharmacologic manipulation of the human complement system will completely eliminate the risk of DCI.

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The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DDH, publ. no. (NIH) 85-23.—*Manuscript received December 1993; accepted March 1994.*

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