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COLD INJURY--ROLE OF LEUKOCYTE AGGREGATION AND
DISRUPTION(U) COLORADO UNIV HEALTH SCIENCES CENTER
DENVER B EISEMAN 02 NOV 82 N00014-81-K-0731

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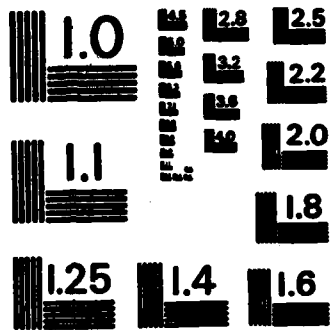
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MICROCOPY RESOLUTION TEST CHART
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University of Colorado Health Sciences Center

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University Hospitals
School of Medicine
School of Nursing
School of Dentistry

4200 East Ninth Avenue
Denver, Colorado 80262

November 2, 1982

AD A 121902

Mr. G. Max Irving
Administrative Contracting Officer
Department of the Navy
ONR Resident Representative
Bandelier Hall West
University of New Mexico
Albuquerque, New Mexico 87131

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Dear Mr. Irving:

→ This Annual Letter Report covers the period November 1, 1981 to November 1, 1982 on the project, Cold Injury--Role of Leukocyte Aggregation and Disruption, in which I am the Principle Investigator. This is Grant No. N00014-81-K0731.

During this period we have achieved the following objectives. *has been perfected.*

→ After prolonged experimentation, ~~we have~~ *perfected* a reliable experimental model. As noted in the preliminary studies, this amounts to anesthetizing adult New Zealand White rabbits (body weight, 1500/2000 gms) and administration of Ringer's Lactate in a right ear vein. The left ear is shaved and a thermprobe connected to a Yellow Springs recorder inserted near the mid-line of the ear 2½ cm from the tip. The ear is marked at centimeter lengths from the tip of the ear to provide accurate level of cold exposure during immersion.

After a control period of 10/15 minutes, an outer container 20 cm in diameter is filled with dry ice and alcohol. An inner container, 7 cm in diameter, is used for immersion of the ear. This is filled with 50% alcohol in Ringer's Lactate. This avoids ice formation and crystals on the ear which in earlier studies interferred with prompt and accurate warming.

The ear is immersed to a level of 5 cm in the cold solution. The temperature of the ear is recorded at 2 minute intervals. When the thermprobe registers -5°C, which characteristically requires 15 to 20 minutes and is repeatable, the outer chamber is emptied of its dry ice, alcohol solution and quickly filled with warm water at 62°C. Characteristically it takes 3 to 5 minutes for the ear temperature to reach 37°C at which time the ear is removed from the immersion unit.

→ The animals are returned to their cages and the ear inspected daily.

Quantitation of the injury is now possible by comparing templates of the ear made before injury and seven days after cold exposure. These are traced on heavy cardboard paper and the outline of the frankly necrotic ear traced on the template one week after cold exposure. Weighing the two heavy cardboard

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templates provides numerical comparison of the area of the ear that has become necrotic due to the cold exposure. This is a highly repeatable figure.

During the last year, the following groups of animals have been studied.

- Group 1. n = 67 No drug treatment. (Controls)
- Group 1A. n = 30 Performed as simultaneous controls during drug treatment during a 3 month period.
- Group 1B n = 10 Simultaneous controls performed during the summer time to confirm the baseline damage at a statistically reliable level.
- Group 2. Nitrogen Mustard (NM₂). n = 13
1.75 mg per kg of body weight NM₂ was administered into the left ear 72 hours prior to cold immersion. Immediately prior to cold immersion, a leukocyte count was performed to confirm that the peripheral white blood count had fallen from 7,000 to 8,000 to 800. Characteristically there was less than 1% of polymorphonuclear cells in the circulating blood.

This study was performed in anticipating that Neutropenia might protect against cold injury.

- Group 3. Dimethylsulfoxide (DMSO). n = 19
4 gm per kg of DMSO in 24 ml of physiologic saline is started in the right ear prior to cold immersion and continued for three hours at the rate of 8 ml per hour following cold exposure. This provides 750 mg of DMSO per hour to a 1500 gram animal.
- Group 4. Dimethylthiourea (DMTU). n = 10
A bolus of 500 mg per kg DMTU in 10 ml of saline is given intravenously 15 minutes before cold exposure and the constant infusion of 500 mg per kg in 24 ml of saline started 15 minutes prior to cold exposure. The infusion is at the rate of 8 ml per hour and continued for 3 hours. This provides an infusion rate of 0.166 grams of DMTU per kilogram of body weight per hour.
- Group 5. Oral Allopurinol. n = 9
Because Allopurinol is an inhibitor of xanthine oxidase which, in turn, is necessary for the production of superoxide, this drug was administered to determine whether it was protective. The animals were given 50 mg/kg of Allopurinol by mouth daily x 3, prior to cold exposure.
- Group 6. Allopurinol + DMSO. n = 37
Oral Allopurinol was given as in the group above for 3 days prior to cold exposure and DMSO administered intravenously immediately prior to cold exposure and for three hours thereafter as in Group 3 above.

- Group 7. Allopurinol Intravenous. n = 10
The sodium salt of Allopurinol was administered as a 50 mg/kg I.V. bolus before freezing and another 50 mg/kg I.V. bolus during the rewarming period in the opposite ear. It was noted that this highly alkaline material required additional anesthesia in the animals, presumably because it irritated the vein. To be certain that the additional anesthesia did not interfere with judging tissue loss from cold, the time required to reach -5°C was recorded in the animals receiving intravenous Allopurinol, as well as in the controls. There was no difference in time of cold exposure.
- Group 8. Allopurinol Intravenous + DMSO + Oral Allopurinol for two days post-freeze. n = 16
As in Group 7 above, Allopurinol was given intravenously along with a DMSO infusion prior to and for three hours after cold exposure but in addition, oral Allopurinol was continued for two days following cold exposure.
- Group 9. Intravenous Allopurinol + DMSO. n = 9
Without prior drug treatment, a bolus of 50 mg/kg Intravenous Allopurinol was given prior to freeze and DMSO and another bolus of Intravenous Allopurinol were started upon rewarming.
- Group 10. Nitrogen Mustard + DMSO. n = 12
Nitrogen Mustard was given as in Group 2 and DMSO given as a three hour infusion as in Group 3.
- Group 11. Imidazole. n = 9
Imidazole is a thromboxane synthetase inhibitor which is alleged to inhibit permeability edema. Imidazole was administered as a 25 mg/kg per hour intravenous infusion starting 30 minutes prior to freeze and continued for one hour.
- Group 12. Acetylcysteine. n = 11
Acetylcysteine binds free radicals such as $\cdot\text{OH}$ and was administered as a 150 mg/kg loading dose and as a continual I.V. infusion of 20 mg/kg/hr. for 3 hours. At the end of the I.V. infusion, another bolus of 100 mg/kg I.V. was given.
- Group 13. DMSO local on rewarm. n = 14
After the ear was frozen, it was removed to a DMSO bath and warmed to 37°C .
- Group 14. Colchicine. n = 8
Colchicine inhibits mitosis in leukocytes and possibly inhibits chemotaxin. A bolus of 0.1 mg Colchicine was injected I.V. 15 minutes prior to freeze and another bolus of 0.1 mg Colchicine I.V. upon rewarming.
- Group 15. Allopurinol + DMTU. n = 12
DMTU as in Group 4 and Allopurinol as in Group 5.
- Group 16. Allopurinol PO before freeze and DMSO at rewarm. n = 11
50 mg/kg Oral Allopurinol was given just before freeze and intravenous DMSO infusion as in Group 3 was started during rewarming.

Group 17. Chemotaxin. n = 4

Four rabbits with no treatment had their ear frozen and blood samples were drawn from the frozen ear at various intervals and the chemotaxins measured.

Group 18. Complement (in progress).

Group 19. Superoxide Dismutase (in progress).

The findings from these studies will be detailed in the formal report but can be summarized as follows:

1. The animal model is accurate for quantitating protection from cold injury.
2. A combination of Allopurinol and DMSO affords significant protection.
3. Neither drug alone is as effective as the combination.

Future studies are designed to determine protection afforded by prostaglandin and prostaglandin inhibitors. Superoxide dismutase is also being investigated. We are encouraged by these studies which for the first time provide solid evidence for protection against cold injury by pharmacologic agents given to an animal prior to rewarming.

I hope this letter provides the information that you wanted as an informal progress report.

Sincerely,

Ben Eiseman, M.D.
Professor of Surgery
Department of Surgery

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