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BRAIN MICROCIRCULATION
OBSERVED IN VIVO AFTER TRAUMA

Annual Report

William I. Rosenblum, M.D.

May 1976

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Cerebral vessels are traumatized and studied for thrombus formation, diameter change, and reactivity to pharmacologic agents. In addition brain is traumatized and undamaged vessels studied, adjacent to the damaged brain. Thus the effects on vessels of brain trauma, is separated from the effects of trauma to the vessels themselves.		

Brain Microcirculation Observed in Vivo After Trauma

Annual Report DAMD 17-76-C-6020

During the past 7 months we have made substantial progress. The significance of our data to the overall problem of head trauma is explained at the end of this report. All our studies to date utilize the UV light and intravenous fluorescein to create an injury to the vessel which induces platelet aggregation. As explained in our original protocol this injury mimics that induced by lasers in combination with intravenous dyes. We utilize vessels on the surface of the exposed mouse brain. Electron microscopic studies of damaged vessels during this investigation showed us endothelial denudation in minute foci, like those only recently reported for the laser-dye model in other microvascular beds. We have acquired the equipment to measure the intensity of our light at the focal plane and keep this constant from day to day. Light intensity is $19-20 \times 10^3$ $\mu\text{watts/cm}^2$. Our EM studies showed no damage to red cells by this light in the presence of dye.

Our data thus far utilize the time between the onset of illumination and the appearance of the first platelet aggregate as the parameter to be quantified. This is measured through the microscope at 110X. As pointed out in the original protocol, the use of UV light and fluorescein provides our system with a unique advantage, namely that the fluorescein produces fluorescence of the aggregates. This in turn provides a sharp endpoint for judging the appearance of first aggregate. Thus far we have performed experiments on several hundred mice with the following results. In the summary that follows the experimental data in some cases, represents only a portion of the studies actually performed with identical results.

Because the time to first aggregate in the arterioles may be dependent upon the interval between operation (craniotomy) and the onset of the light, we have scrupulously controlled this factor, so that for any individual study, this interval is identical for every animal, both experimental and control. The interval used for one study may differ from that used in another, depending upon the requirements of the particular experiment. To eliminate any possible effects of uncontrollable differences between groups of mice received at different times or housed for different times, control and experimental mice were alternated on any given day so that experimental data and control data were always gathered from the same group of animals.

1. Aggregation always occurred in venules before arterioles. This will be obvious from the data below, and conforms with current notions concerning the inverse relationship of red cell velocity and tendency for platelet aggregation. However, this result may also reflect a difference in the properties of venular and arteriolar endothelium, at least in this vascular bed.
2. Anesthetic effects platelet aggregation. Pentobarbital retarded aggregation. This conforms to in vitro data already in the literature.

	<u>Time (Sec) to First Aggregate</u>	
	<u>Arteriole</u>	<u>Venule</u>
Urethane (N=20)	33 ± 7*	22 ± 5
Pentobarbital (N=20)	40 ± 10	30 ± 5
	p<.03	p<.001

*In this and all subsequent tables data is Mean ± S.D. A students "t" test is used in comparisons. Vascular diameters average approximately 30-40 microns in each experiment, and vessels are selected so that mean diameter in the experimental group is the same as mean diameter in the comparison control group.

In all experiments below urethane was the anesthetic unless otherwise noted.

3. Aspirin retards aggregation. This effect has been described in test tubes, but rarely in the microcirculation of living animals. It has been reported only once before for cerebral vessels - in a paper in French which offers an incomplete description of the work. The data is important in view of the interest in aspirin as a therapeutic or prophylactic agent for cerebral thrombosis - a phenomenon which must occur in brain injury.

	<u>Time to Aggregate</u>	
	<u>Arteriole</u>	<u>Venule</u>
Aspirin 100 mg/kg i.p. (N=10)	102 ± 62	25 ± 5
Control (N=10)	41 ± 7	21 ± 4
	p<.01	

NOTE: Light stimulus began 60 minutes after aspirin or control (vehicle). The difference between aspirin and control group is also significant with a nonparametric test. The aspirin only affects arteriolar aggregates. This may point to a difference between arteriolar and venular endothelium in this vascular bed.

4. The effect of aspirin is dose dependent and time dependent.

	<u>Time to Aggregate</u>	
	<u>Arteriole</u>	
Aspirin 25 mg/kg 60 min (N=20)	43 ± 10	} p<.05
100 mg/kg 30 min (N=20)	52 ± 17	
100 mg/kg 60 min (N=20)	102 ± 62	
	p<.05	

5. Heparin had no effect on the time to first aggregate in arterioles. We are currently investigating heparins effects on venules. The negative data from arterioles is expected since heparin has no direct action on platelet aggregation, and our EM observations show that when aggregation is first initiated no fibrin has yet been formed.

6. Studies of summative effects of light stimulus.

We have injured the vessels in 2 ways: either by continuous exposure to light or by intermittent exposure (10 seconds of light every two minutes). In either case fluorescein is in the circulatory system. In the latter case a second fluorescein injection was required half way through the experiment in order to maintain dye concentration during the prolonged experimental period. The data indicate that the total exposure to light required to induce platelet aggregation, is the same whether the light is continuous or intermittent.

	<u>Exposure Time to First Aggregate</u>	
	<u>Arteriole</u>	<u>Venule</u>
Continuous exposure (N=20)	41 \pm 7	21 \pm 4
Intermittent exposure (N=20)	39 \pm 8	17 \pm 2

7. Miscellaneous Studies: We have shown that the effect of aspirin is not correlated with alterations in blood pH or CO₂, produced by aspirin. Further evidence that the aspirin effect is not due to an effect of aspirin on arterial CO₂ or pH comes from a study of 20 controls and 20 aspirin treated animals, where 25 mg/kg aspirin was given i.p. and light exposure was for 10 seconds every 2 minutes. In this particular study the blood pH and CO₂ were identical yet aspirin still exerted its effect on time to first aggregate in the arterioles (46 \pm 9 sec vs. 39 \pm 8 sec in control, p<.03).

We have shown that the time to first aggregation is not correlated with mean arterial blood pressure over a range of 50-120. Pressures were measured with a tail cuff. At least 4 mice were used in each pressure decade over the stated range. Pentobarbital was the anesthetic.

SUMMARY & DISCUSSION

The preceding studies indicate the value of our technique as means for evaluating effects of potential therapeutic agents on platelet aggregation due to vessel injury. They also indicate a parallel between drug activity in our in vivo system, and drug activity reported by others in vitro.

It should also be noted that we have repeated earlier studies of ours, and confirmed that the UV light by itself - with the same filters used to excite the fluorescein, fails to produce platelet aggregation. Moreover, when fluorescein is present, the aggregates appear only in the vessels illuminated by the UV light and not in the vessels in non illuminated areas of the brain surface. When no dye is used no damage occurs. Thus we confirm our original impression that platelet aggregation is dependent upon local vascular damage in the illuminated area of vessels containing dye.

The evidence that aspirin preferentially protects arterioles rather than venules is of interest, since studies of aspirins effect on large vessel thrombosis have indicated that aspirins principle action would be on arteries rather than veins. This has been conventionally explained on the basis of the relative unimportance of

platelets in venous thrombosis. However, in our study platelets are the essential, and virtually only, component of the initial aggregate in both arterioles and venules, yet aspirin still shows a preferential action on arterioles. This suggests that the differential effect of aspirin may depend on some differential property of arteries and veins other than the relative importance of platelets in the thrombi within these vessels.

Finally, all the data given above utilized time to first aggregate as the quantified parameter. We have also performed extensive parallel studies in which time was monitored until flow within a vessel was entirely stopped by the accumulating platelet mass. To date, where a statistically significant difference between groups has appeared, it has paralleled the difference in time to first aggregate. For example, not only did pentobarbital prolong time to first aggregate, but it also lengthened the time it took for flow in an arteriole to be totally stopped by aggregates (143 ± 48 sec compared with 108 ± 33 sec in a urethane control group, $N=20$ each group, $p < .02$). This parameter - time to flow stoppage - may prove useful in further studies.

Significance of Data to Problem of Brain Trauma and Future Plans

Vascular damage always occurs in brain trauma and may greatly augment the effects of the trauma. Moreover it is possible that vascular damage can occur in one of two ways: by direct damage to the vessel or by a release of materials from injured brain which could then adversely effect adjacent vessels. Our original proposal had 2 parts and was planned for a multiyear period. One part was an investigation of the effects of trauma on the vessels themselves. The other was an investigation of the effects of damaged brain on adjacent, undamaged vessels. These types of damage cannot be separated in the conventional models of trauma. The first year we have concentrated on the effect of direct vascular damage. Although, we have utilized just one model of damage (light + dye) the model induces platelet aggregates in damaged vessels + provides the visual advantage of fluorescing aggregates. Moreover, the failure of heparin to influence the model, and the inhibiting influences of both pentobarbital and aspirin, are identical to the effects observed on platelet aggregation produced in pial microvessels by other workers using techniques of vessel "pinching" to produce damage (Honour & Mitchell Br J Exp Path 45:75-87, 1964; Born GVR and Philip RB Br J Exp Path 46:569-576, 1965; Gautier et al Ann Med interne 123:849-852, 1972). Thus our model appears to provide data that is not specific to the particular form of injury we are using, and this of course is a great advantage. We have demonstrated that the model provides a reliable in vivo test system for drugs known to have anti platelet aggregate activity in vitro. We will perform additional tests with drugs (e.g. indomethocin) which have such activity and may be clinically useful in treating platelet aggregates or preventing their formation or propagation, whatever the nature of the trauma initiating them.

We now intend to investigate the effects on the light-dye model, of mechanical injury to brain substance adjacent to the vessel. In this way we will be able to evaluate a possible effect of materials released from injured brain, on aggregation occurring in vessels injured separately by our already established model of vessel injury. Naturally, we shall also observe whether brain injury alone can elicit aggregation in adjacent vessels. If an effect of brain injury is observed on adjacent vessels, we will be able to extend our drug studies to include evaluation of prophylaxis and treatment of the adverse effects of injured brain on adjacent vessels. It is thought that these studies of brain injury will encompass the major part of the second years work, and will precede studies of vessel injury using noxious stimuli other than dye and light.

Vascular trauma and/or release of materials from injured brain may produce altered vascular diameter (e.g. spasm) in addition to platelet aggregates. Just as aggregation may be harmful in brain injury, so may vasospasm. The present studies have not involved measurements of diameter. The utilization of platelet aggregation as a parameter for study has proved so fruitful that we have not had the opportunity to study diameter changes as well.

As indicated above, we plan to continue our emphasis on aggregation as the parameter of interest in the coming year. However, the protocol for one year renewal, which accompanies this annual report, is written once again, as if it were a multiyear study, and so outlines also an investigation of the effects of brain and vessel injury on vessel diameter. In addition it outlines even more distant studies of models other than light-dye which permit vascular damage without damage to adjacent brain (for example pinching of the vessel).

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