RETROSTERNAL HEMORRHAGE: AN
EXPERIMENTAL MODEL FOR STUDY
OF LYMPHATIC LEAKAGE

S. Levine, A. Saltzman

Department of Pathology, New York Medical College, Valhalla, New York,
USA

ABSTRACT

Courtice and colleagues observed that blood injected into the peritoneal
cavity of rats occasionally leaked from retrosternal lymphatics. The present work
shows that this leakage is determined by volume as well as dose of inoculum. The
uniform occurrence of visible retrosternal hemorrhage after injection of diluted
blood suggests its use as a model for lymphatic leakage. Leakage was prevented
when the blood was instilled during the healing phase of a chemical peritonitis.

In 1953, Courtice, Harding and Steinbeck published a classic paper in
which they showed that the absorption of red blood cells (RBC) from the peri-
toneal cavity into the parasternal lymphatics was rapid, especially in the rat
(1). They observed that "During the passage of the red cells along the parasternal
lymph trunks, some of the red cells occasionally leaked out of the lymph
tributaries of these trunks to give an extravasation of red cells beneath the
pleura or into the pleural cavities." In the present work, we have defined the
conditions to convert this "occasional" extravasation into a constant occurrence
so that it might be used for further studies on lymphatic leakage.

MATERIALS AND METHODS

Lewis rats of either sex, 220-350g
(Harlan Sprague Dawley, Inc., Indiana-
polis) were maintained in hanging wire
mesh cages on Purina Rodent Show 5001
and tap water ad libitum. They were
fasted overnight before intraperitoneal
(IP) inoculations in order to avoid
accidental penetration of the gastrointesti-
nal tract. Rat or mouse blood was defi-
brinated by swirling over wooden sticks.
Whole defibrinated blood or washed
RBC, or dilutions prepared with sterile
saline, were injected IP without anes-
thesia. The recipients rats were held verti-
cally, head down, during injection, and
then they were rapidly rotated three
times to ensure wide distribution of the
inoculum.

In the experiments to determine the
effects of prior inflammation, a sterile
chemical peritonitis was produced by IP
injection of 50ml/kg sodium hypochlorite
(NaOCl, household bleach) diluted 1:100
in saline, as described previously (2-4).

Rats were sacrificed by exsanguina-
tion from neck vessels while under ether
anesthesia. The abdomen was opened in
the midline. Residual fluid was absorbed
into a pre-weighted 4 inch square gauze
gauze sponge which was pushed into all parts
of the peritoneal cavity including lumbar
gutters, pelvis and the subdiaphragmatic
space. Testes and fluid in the scrotum
were pushed into the abdominal cavity by external pressure aided by gravity. While the sponge was being weighed, the abdomen was opened more widely with a horizontal incision, and the absorption of remaining fluid accomplished with a second gauze sponge. The second sponge usually absorbed only 1/10 to 1/20 as much as the first sponge and it left the peritoneum free of any visible blood. The procedure was carried out rapidly in order to minimize losses from evaporation. In each of two normal, uninoculated rats, 0.35g fluid was recovered in this manner; this "blank" value was subtracted from all recovery data. The same procedure was carried out in two rats that had been given NaOCl one week previously but no further inoculation. An almost identical "blank" value was obtained. The volumes of inoculated fluids were converted to grams (specific gravity of blood 1.05) and residual fluid was expressed as percent by weight of inoculum recovered in the sponges.

After removing residual blood, the thorax was opened and hemorrhages in the retrosternal area were noted and scored from 1+ to 4+, where 1+ was a minimal lesion, only a few mm in size, and 4+ was a maximal lesion covering a few cm². The parathymic nodes were weighed fresh.

Nodes and sternums were fixed in Bouin's fluid, the latter were decalcified, and both tissues were embedded in paraffin, sectioned and stained with hematoxylin-cosin-phloxine.

RESULTS

Retrosternal hemorrhages were observed 3, 5, 24, and 48 hours (but not 1 hour) after IP inoculation of blood. The hemorrhages usually occupied a triangular area, the apex behind the middle or upper third of the sternum and the base near the attachment of the diaphragm (Fig. 1). Within this area, the hemorrhages were blotchy and irregular or they followed one or several costal cartilages. They never extended so far laterally as to reach the bony ribs. The term "retrosternal" was not strictly correct because a narrow vertical midline strip immediately behind the sternum was usually spared. The hemorrhages were frequently asymmetrical or even unilateral but without predilection for one side or the other.

Microscopically, the hemorrhages were subpleural or in the layer of fat that intervened between the thin strip of subpleural muscle and the costal cartilages with attached intercostal muscles. The main retrosternal blood vessels and lymphatics were located in this same layer of fat. The retrosternal lymphatics were filled with blood, presumably derived from the IP inoculum. In the midline, the epimysium of the subpleural muscle blended with the periostium of the sternum, which explained the inability of hemorrhages to penetrate to this midline site. Rats killed 8 days after inoculation had only hemosiderin deposits to attest to the occurrence of retrosternal hemorrhage.

Courtice et al had injected 20ml/kg of whole heparinized blood or washed RBC and observed occasional hemorrhages (1). In our rats, 10ml/kg of defibrinated blood produced retrosternal
hemorrhages in all subjects but some were mild. Uniformly severe hemorrhages were obtained when the same dose of blood was injected after dilution 1:5 in sterile saline (Table 1). The importance of a large volume of inoculum was demonstrated even more clearly when a small suboptimal dose of defibrinated blood (2ml/kg) was injected with or without dilution (Table 1). Washed RBC reconstituted with saline to original hematocrit and then diluted 1:5 produced the same results as the corresponding doses of whole blood recorded in Table 1. In subsequent experiments, 10ml/kg of blood or reconstituted RBC was the routine dose and it was diluted 1:5 in saline before injection. Hemorrhages were observed from mouse as well as rat RBC (Table 2) and rat blood from a different strain (LBN F hybrids) was also effective. Rat blood injected into Fischer 344 rats produced hemorrhages similar to those in the Lewis rats that were used in all the other experiments.

Lymphatic absorption of dyes, metal powders, oils and cells is increased during the healing phase of a chemical peritonitis (2-4). Therefore, rats were given NaOCl IP one week before injection of blood or RBC. The NaOCl did not cause any obvious indisposition of the rats but it left them with fibrosis of liver and spleen surfaces and various degrees of obliteration of the greater omentum (2). The NaOCl pretreatment prevented retrosternal hemorrhages following IP blood (Table 2).

The effect of NaOCl on absorption of the inoculum was studied (Table 2). As a baseline for this study, two rats were inoculated with blood and immediately anesthetized and exsanguinated. Niney percent of the inoculum was recovered from their peritoneal cavities. The missing 10% may have been absorbed during the process of obtaining the peritoneal contents and/or may have evaporated during the weighings. Rats killed at intervals after inoculation revealed a progressive diminution of peritoneal content but there was a wide spread of individual values and no clear difference between control and NaOCl treated rats.

Eight additional rats were given NaOCl 3 days instead of 7 days before challenge with the usual dose of rat blood or RBC. None of them had retrosternal hemorrhages when necropsied 3, 5, or 24 hours later in contrast to the controls. In an effort to overcome this inhibitory effect, two rats were given 50ml/kg of a rat RBC suspension (five times the usual dose) one week after NaOCl treatment, but to no avail, whereas the control rats (no NaOCl) developed the usual retrosternal hemorrhages.

<table>
<thead>
<tr>
<th>Concentration of Blood</th>
<th>Volume ml/kg</th>
<th>Total IP Dose of Blood ml/kg</th>
<th>Retrosternal Hemorrhages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>10</td>
<td>4,2,2</td>
</tr>
<tr>
<td>1/5</td>
<td>50</td>
<td>10</td>
<td>4,4,4,4</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0,0,0,0</td>
</tr>
<tr>
<td>1/5</td>
<td>10</td>
<td>2</td>
<td>1,0,0,0</td>
</tr>
<tr>
<td>1/25</td>
<td>50</td>
<td>2</td>
<td>4,1,1, tr</td>
</tr>
<tr>
<td>1/25</td>
<td>10</td>
<td>0.4</td>
<td>0,0</td>
</tr>
</tbody>
</table>

*Rat blood was defibrinated. Concentration of '1' denotes undiluted blood. Note that concentration x volume = dose.

Scored from 1 to 4 (see text) in individual rats. 'tr' = trace.

---

Permission granted for single print for individual use.
Reproduction not permitted without permission of Journal LYMPHOLOGY.
Table 2
Healing Peritonitis Prevents Retrosternal Hemorrhage

<table>
<thead>
<tr>
<th>Hours After IP Blood</th>
<th>Retrosternal Hemorrhages&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Residual IP Blood, %&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>After NaOCl</td>
</tr>
<tr>
<td>0 (rat blood)</td>
<td>0,0</td>
<td>ND</td>
</tr>
<tr>
<td>3 (rat RBC)</td>
<td>4,2,2</td>
<td>0,0</td>
</tr>
<tr>
<td>4 (rat RBC)</td>
<td>3</td>
<td>ND</td>
</tr>
<tr>
<td>5 (rat blood)</td>
<td>4,3</td>
<td>1,1,0,0</td>
</tr>
<tr>
<td>5 (rat blood)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4,3</td>
<td>1,1</td>
</tr>
<tr>
<td>6 (mouse RBC)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>24 (rat RBC)</td>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>24 (rat blood)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4,4</td>
<td>1,0</td>
</tr>
<tr>
<td>24 (mouse RBC)</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

<sup>a</sup>Scored from 0 to 4+ (see text) in normal rats with healing peritonitis due to injection of NaOCl one week previously. ND = not done.

<sup>b</sup>Weight of fluid recovered on gauze.

<sup>c</sup>10ml/kg of whole blood or washed RBC reconstituted to original hematocrit, diluted 1:5 for injection.

<sup>d</sup>Female recipients; all others were male.

**DISCUSSION**

The original observations of Courtice, Harding and Steinbeck on retrosternal hemorrhages (1) were incidental to a detailed study of the absorption of RBC from the peritoneal cavity (1,5). The escape of RBC was considered to be basically similar to the escape of plasma into the retrosternal tissues and pleural cavity after IP injection of plasma (6,7). The same points were made without further elaboration in the monographs published 3 and 17 years later (8). There is considerable interest in the study of the permeability of lymphatic vessels in relation to function and ultrastructure (9-11), and special methods and approaches have been developed (12,13). Nevertheless, we have been unable to find any further application of this extremely simple way to produce and study leakage of cells from lymphatics, nor have we found any mention of the phenomenon in various monographs on lymphatics (10,14). The explanation may be the relative infrequency of its occurrence (1). However, the uniform occurrence of hemorrhages when we injected large volumes of diluted blood should make it useful as an experimental model.

It should be noted that increasing the volume of fluid or the number of particles inoculated IP has been shown to increase the amount absorbed in other experiments (15,16). We studied the effect on retrosternal hemorrhages of a subsiding peritonitis because of previous demonstrations that inflammation in the healing phase increased the absorption from the peritoneal cavity of various particulates (2-4). Courtice et al had attributed the leakage to partial obstruction within the para-thymic lymph nodes and subsequent damming back of lymph flow (1). If this is valid, the slight enlargement of these lymph nodes following NaOCl peritonitis might have prevented the obstruction and thereby prevented the leak. Alternatively, the peritonitis might have prevented leakage by reducing the permeability of the lymphatics draining the inflamed cavity, but there is no evidence for this hypothesis. In fact, increased permeability has been noted in lymphatics draining a site of inflammation (albeit of a different location and type) (9). Nevertheless, the lymphatic vessels are not merely passive conduits (17); they are the actual site of leakage and they may be directly involved in the inhibition of leakage by NaOCl.
The data of Table 2 provide a hint of a third explanation: NaOCl might decrease or slow the absorption of blood from the peritoneal cavity. Decreased absorption of amorphous glass particles has been found one day after infection of peritonitis by turpentine (16). Unfortunately, the amount of residual blood that was found was so variable that no conclusion could be drawn (Table 2). Perhaps the inhibition of leakage of NaOCL involves some combination of retarded absorption and structural changes in the draining lymphatics and/or in the draining lymph nodes.

ACKNOWLEDGEMENTS

Supported by a research grant from the National Institutes of Health NS 22261. We are grateful for assistance from John Coleman and Lorraine Ostrubak, and for the photography by John Eisner.

REFERENCES


Seymour Levine, M.D.
Department of Pathology
New York Medical College
Basic Science Building
Valhalla, NY 10595 USA