Autoradiographic Detections of \(^{111}\)Indium-Labeled Platelets in Brain Tissue Sections

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SUMMARY Various techniques for detection of blood platelets in tissue section were evaluated in an incremental air embolism model of ischemia in order to further investigate the accumulation of platelets in brains subjected to cell-damaging ischemia. The experimental animals were dogs anesthetized with alpha-chloralose. A new autoradiographic technique was devised that allows precise localization of \(^{111}\)Indium-labeled platelets in brain sections. The technique is described in detail along with some effects of the label on platelet function and behavior. The technique can be performed in conjunction with \(^{14}\)C-iodoantipyrine autoradiographic measurement of blood flow without causing mutual interference or other forms of interaction. This permits the simultaneous investigation of local blood flow and deposition of platelets.

THE ROLE OF BLOOD PLATELETS in the pathogenesis of many diseases is difficult to assess accurately because it is generally difficult to detect platelets in tissue using routine histological techniques.\(^1\) In order to determine whether ischemic brain injury in a canine model of multifocal ischemia\(^2\) is associated with accumulation of platelets, we developed a new method for identifying platelets in brain sections; this method also permits correlations with local blood flow. Initially, several reported techniques for detecting platelets in tissue sections were evaluated. Attempts were made to identify platelets by means of fluorescent microscopy after labeling the platelets with mepacrine.\(^3\) We noted several drawbacks, however, especially in the use of mepacrine. Mepacrine alters the accumulation and release of serotonin,\(^4\) inhibits phospholipase A\(_2\),\(^5\) and also inhibits platelet aggregation.\(^6\) In concentrations as high as 100 \(\mu\)mol, we found ADP-induced aggregation of platelets was completely inhibited by mepacrine labeling. Although mepacrine-labeled platelets could be detected within large vessels of tissue sections by their characteristic yellow-green fluorescence, we were unable to identify any postischemic accumulation of platelets in damaged zones of the brain by using this method.

We also tried labeling platelets by the uptake of radioisotopically-tagged serotonin, a method used by other researchers.\(^7,8\) ADP-induced aggregation was slightly reduced by the serotonin labeling process. When \(^{3}\)H-serotonin, freed of impurities by thin layer chromatography (TLC), was incubated with platelets and infused, no accumulation of radioactivity was demonstrated in an autoradiograph of the hemisphere injured by ischemia. When \(^{3}\)H-serotonin containing degradation products (three major compounds by TLC) was incubated with platelets and infused, focal concentrations of radioactivity appeared on autoradiograms clustered around large blood vessels in the gray matter of the injured hemisphere. The same phenomenon was observed on autoradiograms when a platelet-free solution of \(^{3}\)H-serotonin containing the same degradation products was infused into animals subjected to brain ischemia. The findings suggested that the autoradiographic images were caused by the labeled serotonin breakdown products that had penetrated a damaged blood-brain barrier. We concluded these techniques for detection of platelet accumulation in sections of the ischemically-damaged brain were flawed by important limitations. As a result, we embarked on an effort to develop an improved method for autoradiographic detection of platelets.

Other workers have found that platelets can be efficiently labeled with \(^{111}\)Indium-oxine,\(^9,10\) and that this isotope is suitable for autoradiography.\(^11,12\) Furthermore, the 2.8-day half-life of \(^{111}\)Indium offers an opportunity to carry out double-label autoradiography in conjunction with \(^{14}\)C-iodoantipyrine. We anticipated that this type of combined autoradiography should permit correlations between focal accumulation of platelets and local blood flow.

Materials and Methods

This technique for autoradiographic detection of \(^{111}\)Indium-labeled platelets in conjunction with \(^{14}\)C-iodoantipyrine autoradiographic blood flow measurement\(^13\) was developed in a previously described model of focal cerebral ischemia.\(^14\) Ischemia was produced on one side of the brain by the incremental injection of small volumes of air through the ipsilateral internal carotid artery of dogs. The frequency and volume of the air injection was determined by the effect of the embolic air on the cortical somatosensory evoked response (CSER), an electrophysiologic index of neuronal function that is quantifiable. The contralateral hemisphere was kept intact as a control.

Preparation of the Animals

Male mongrel dogs weighing from 8–15 kg were premedicated with xylazine (ROMPUN) 1.1 mg/kg and atropine 0.05 mg/kg subcutaneously. This was followed by an initial intravenous dose of alpha-chloralose 80 mg/kg and incremental doses as necessary. Anesthetized animals were ventilated mechanically through an endotracheal tube with a BIRD respirator. End-tidal PC\(_2\) (Beckman LB2) and PO\(_2\) (Beckman OM11) were monitored continuously to help maintain normal levels of PC\(_2\) and PO\(_2\); rectal temperature was

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measured and maintained at 38°C with heating pads and infrared light.

A catheter inserted proximally in a femoral artery permitted measurement of systemic arterial blood pressure by means of a STATHAM strain gauge and a GOULD polygraph. Two catheters were placed in the other femoral artery: one was directed proximally into the aorta and used to collect arterial blood for platelet labeling, arterial blood gases and pH measurements; the other was inserted into the distal femoral artery. When these two catheters were later joined through a Y-connector, arterial flow to the leg was externalized and this permitted rapid sampling of arterial blood for the 14C-iodoantipyrine autoradiographic blood flow measurement. A right ventricular catheter was placed via the right femoral vein to facilitate induction of rapid circulatory arrest by intracardiac injection of saturated potassium chloride. This was a requisite step for the iodoantipyrine blood flow technique. Another venous catheter placed in the left femoral vein allowed infusion of various solutions: saline, drugs, labeled platelet suspension and 14C-iodoantipyrine. On one side, the internal carotid artery was exposed and catheterized with a PE50 tube filled with physiological saline; the catheter permitted direct, selective injections into the cerebral circulation.

The dogs were placed in a Kopf stereotactic apparatus. The recording electrode was inserted into a hole drilled over the sensorimotor cortex of the side to be injured, and the reference electrode was inserted in the nasal bones at their distal extreme. The potentials from the recording electrodes were initially led to a NICOLET HGA-100 preamplifier with a 25-10 kHz bandwidth and a 10^6 gain. The output signal was fed to a TEKTRONIX 5110 oscilloscope. The CAT output was recorded on a Hewlett-Packard 7045A X-Y recorder. Sixty-four evoked responses were averaged to obtain each recorded response.

The stimulating needle electrodes were inserted percutaneously such that they straddled the median nerve in the upper foreleg contralateral to the hemisphere to be embolized. A square wave stimulus of 300 µsec duration at a rate of 1.1/sec was led through a Grass Model PSIU photoelectric stimulus isolation unit to the implanted needle electrodes. The strength of the stimulus was adjusted to cause a maximal somatosensory evoked response.

Animal Experiments

Baseline CSERs were recorded, and 50–100 µl of room air were injected into the side to be injured through the catheter in the internal carotid artery, and flushed in with 500 µl of saline. After 2–3 min, another CSER was recorded. If the response was suppressed to approximately 10% of control P₁ – N₁ amplitude, no more air was infused. If the CSER was only suppressed partially, another 20–50 µl of air was injected. Subsequently, the periodic re-emergence of a growing CSER was resuppressed by the infusion of 20–50 µl of air. This cycle of alternating emergence and ischemic suppression of the evoked response was continued for 1 hr.

The 1-hr ischemic period was followed by a 4-hr recirculation period during which blood gases and blood pH (Corning 165/2 pH/Blood Gas Analyzer), blood pressure, and rectal temperature was monitored. Twenty ml of 111Indium-labeled platelet suspension were re-injected into the systemic circulation via the femoral vein catheter at the beginning of the recirculation period. At the end of the 4-hr recovery period, a 14C-iodoantipyrine blood flow study concluded the experiment. Essentially, this required intravenous infusion of 50 µCi per kg of 14C-iodoantipyrine for 1 min and serial sampling of arterial blood every 5 sec. An intracardiac injection of saturated potassium chloride induced cardiac arrest and thus terminated the experiment. Local blood flows were calculated as previously described by Sakurada et al from 1) tissue concentrations of 14C-iodoantipyrine, which were determined autoradiographically, and 2) concentrations of the tracer in the sequential arterial blood samples.

Preparation of the Labeled Autologous Platelets

The technique used was derived from a procedure described by Scheffel et al. Blood collection and processing techniques were standardized to optimize the yield and activity of the labeled platelet preparations. All labeling processes were performed at room temperature, and both platelet-rich plasma (PRP) and platelet-poor plasma (PPP) were kept in closed tubes to avoid an increase in pH due to the escape of carbon dioxide. To minimize platelet activation during pelleting, only round-bottom centrifugation tubes were used. Platelet buttons were resuspended by gentle, successive aspiration and ejection of the resuspension medium with a plastic disposable pipette. By keeping the tip of the pipette immersed, bubbles were avoided.

A. Blood Collection

Platelets were collected from arterial blood withdrawn from the femoral artery catheter into Anticoagulant Citrate Dextrose solution (ACD — Formula A, Fenwall Laboratories, Deerfield, IL) at a final ratio of 15% v/v. In practice, 102 ml of arterial blood were collected in two plastic syringes; each syringe contained 9 ml of ACD solution and 51 ml of blood. Ringer’s lactate solution (100 ml) was injected immediately after blood sampling to restore the blood volume.

B. PRP Preparation

The content of the two syringes (120 ml) was divided equally into four 50-ml polycarbonate centrifugation tubes, then centrifuged 10 min at 1000 rpm (130 g) (Damon/IEC Division, centrifuge model HN-SII). Such a centrifugation yielded approximately 30 ml of PRP, depending on hematocrit and blood viscosity. The resulting PRP contained approximately 370,000 platelets/µl. A final washing of the 111Indium-labeled
platelets was necessary when the labeling was accomplished in a plasma medium because a significant part of the label remained unbound to platelets at the end of the incubation period. This final washing (which is more fully described below) was performed with PPP obtained after collecting PRP by packing the remaining red cells with a 10-min centrifugation at 2300 rpm.

C. Preparation of the Concentrated Platelet Suspension

To increase labeling efficiency, platelets had to be concentrated in a small volume before incubation with radioactive Indium-oxine. They were protected during their pelleting by acidifying PRP with the ACD solution (addition of an ACD volume equal to 1/20 of the PRP volume). The acidified PRP was then divided into two 50-ml polycarbonate centrifugation tubes and centrifuged in two steps: 10 min at 1800 rpm (580 g) and 10 min at 2200 rpm (870 g). Most "heavy" platelets sedimented with the first spin. Except for 4 ml, both supernatants from the first spin were transferred into two other centrifugation tubes, while the two pellets were resuspended in the remaining 4 ml. The second spin yielded the rest of the platelet suspension. Finally, the acidified PPP (supernatant) was collected and saved, and the pellets were resuspended with the 4 ml containing the platelets sedimented in the first spin. This 4 ml of platelet concentrate constituted the incubating medium.

D. Incubation with 111In-oxine

The isotopic solution was purchased from MEDI-PHYSICS INC. (Emeryville, CA). Each dose of 111In-oxine (1.5 mCi at the time of the experiment) was dissolved in 50 μl of pure ethanol. To avoid a high local concentration of alcohol during the addition of the isotope, these 50 μl were diluted by the addition of 200 μl of 0.9% saline. All of the diluted isotope solution was then added to the 4 ml of incubating medium drop by drop, with a gentle rotation of the tube to disperse the isotope solution rapidly in the platelet concentrate. After 30 min of incubation at room temperature, the labeling was stopped by the addition of the 20 ml of acidified PPP previously saved.

E. Washing of the 111In-labeled Platelets

Before pelleting the labeled platelets, 20 μl of the incubating medium was saved in a small hemolysis tube. Later this sample was analyzed by gamma scintigraphy (PRP1) to evaluate labeling efficiency. The labeled platelets were pelleted by means of the same double centrifugation technique as in the preparation of the platelet concentrate. The radioactive platelet-poor supernatant was discarded except for 20 μl (PPP1) that was saved to evaluate labeling efficiency. The pellets were gently resuspended together in 20 ml of the PPP that was saved at the beginning of the PRP preparation as described above. This suspension was finally drawn into a shielded syringe through a short extension tube.

F. Evaluation of 111In-labeling of Platelets

Labeling efficiency was evaluated by calculating the gamma activity ratio: (PRP1 count – PPP1 count) / PRP1 count. This ratio is a reasonable approximation of the efficiency because the volume of the platelets is small in comparison to the total volume of the platelet suspension.

To quantitate the fraction of 111Indium unbound to platelets at the time of re-injection, a 100 μl sample of the final labeled platelet injection was saved (PRP1) and part of the sample was centrifuged to get the corresponding PPP1. After counting the gamma activity of PRP1 and PPP1 samples (20 μl each), the ratio of PPP1 counts to PRP1 counts was calculated. This ratio indicates the fraction of the 111Indium remaining unbound.

Aggregation Tests

To check the reactivity of the labeled platelets, aggregation studies were performed with an optical aggregometer according to the technique of Born. Aggregation was recorded for at least 3 min.

When compared to PRP prepared by standard techniques from citrated blood (1 volume of 3.8% sodium citrate and 9 volumes of blood), PRP prepared from ACD-anticoagulated blood (15% ACD solution as described above) showed poor reactivity with ADP. This inhibition was due to: 1) a higher citrate concentration of the ACD (final citrate concentration was 17 mmol with ACD, 13 mmol with 3.8% citrate), and 2) a lower pH of the ACD-anticoagulated blood (pH = 7.0 compared to 7.6 with citrated blood). ACD inhibition of aggregation induced by ADP, however, was totally reversible once the platelets were pelleted and resuspended in citrated plasma. Because labeled platelets were harvested from ACD-anticoagulated blood and suspended in ACD plasma, investigation of the aggregability of labeled platelets was only possible when they were resuspended in citrated plasma. This was achieved by means of the two-step centrifugation previously described.

In addition to aggregation studies, suspensions of labeled platelets were observed under a phase-contrast microscope for aggregates.

Survival of 111Indium-labeled Platelets

After the re-injection of labeled platelets, the rate of 111Indium clearance from the blood was monitored by serial blood sampling, and at autopsy, tissue samples from various organs were counted to evaluate platelet sequestration. In these measurements, the influence of variations in blood volume was taken into account. Total blood volume and organ blood volumes were measured by means of 3Cr-labeled autologous red cells that were re-injected shortly after the 111Indium-labeled platelets.

A. 3Cr-labeling of Red Cells for Determination of Blood Volume

Packed red cells obtained after collecting PPP were labeled by 3Cr. One hundred μCi of 3Cr was mixed with 20 ml of packed red cells. 3Cr-sodium Chromate
in a solution of isotonic saline was supplied by NEW ENGLAND NUCLEAR. Thirty min of incubation at room temperature led to labeling efficiencies higher than 95%, which allowed at direct re-injection of the 51Cr-labeled red cells once rediluted with 20 ml of autologous plasma saved at the beginning of the labeling.

B. Determination of the Rate of 111In Clearance from Blood

This investigation was performed by collecting 2 ml samples of arterial blood each half hr. Samples were weighed and counted for both 51Cr and 111In radioactivity using a Packard gamma counter. Results for each animal were expressed relative to the blood sample collected 30 min after the re-injection of the labeled blood cells.

C. Sequestration of 111In-labeled Platelets

Samples of the spleen, liver, and lung were collected during autopsy to detect any abnormal sequestration of labeled platelets by these organs. Tissue samples were weighed and counted for 111In and 51Cr in the same manner as the blood samples. Radioactivity concentrations of each sample were expressed as counts/min/gm of fresh tissue, and multiple samples for each tissue were averaged. In each animal, radioactivity ratios for 111In (tagged to platelets) and for 51Cr (tagged to red blood cells) were determined for each organ relative to the radioactivity of a blood sample collected just before the end of the experiment (tissue to blood ratio). This permitted a comparison of the radioactivity of organs from animals with varying blood levels.

Double Autoradiography of 111In-labeled Platelets and 14C-iodoantipyrine

The brain was removed from all of the animals, and frozen in liquid freon that was maintained at —50 to —60°C by suspension over liquid nitrogen. Later, the frozen brain was cut with a cryomicrotome (American Optical Corp., Buffalo, NY). SB5 x-ray films from Eastman Kodak were incubated with the tissue sections to produce the autoradiograms. The 2.8 day half-life of 111In was particularly suited for double-label autoradiography with the long half-life isotope, 14C. Immediate incubation gave an autoradiographic image of both radioisotopes, while a second delayed incubation yielded an autoradiograph of 14C activity alone (a 10-day delay was sufficient since more than 90% of 111In activity had decayed after this interval). In order to obtain the sole autoradiographic distribution of 111In, preliminary experiments were conducted to find a treatment of the tissue sections which would remove 14C-iodoantipyrine without disturbing the 111In tagged to the platelets. We found that methanol was a suitable washing agent for this purpose. Because it was regarded as technically impractical to change the adjustment of the section thickness from 40 to 20 µm in alternating fashion during brain sectioning, autoradiographic blood flow studies were also performed with 40 µm sections.

Consequently, two sets of consecutive 40 µm coronal sections of the brain were processed. One section was washed, stained and immediately incubated for 111In autoradiography. Washing and staining were performed by sequentially dipping the sections in the following solutions:

- 10 min in 100% methanol (elute 14C-iodoantipyrine)
- 5 min in 100% methanol (rinse)
- 5 min in 0.1% Eosin B in methanol-water 50/50 (v/v) (stain)
- 5 min in 100% methanol (destain the grey matter)
- dry the tissue sections at room temperature.

The second section was saved for a delayed incubation, which revealed only the 14C concentration on the autoradiogram.

The Relation Between Apparent 111In Accumulation in Tissue Sections and Increased Cerebral Vascular Space

To determine whether the increase of 111In-radioactivity in the injured hemispheres of embolized animals was due to an increase in the vascular space (vasodilation, microhemorrhages), samples of the injured side of the brain were counted for both 111In and 51Cr radioactivity. Then, they were compared with corresponding samples of the control side. Once the tissue sectioning on the microtome was completed, 50—100 mg brain samples were cut from the tissue blocks and weighed in test tubes. Counting of these samples was performed as described above for the spleen, lung, and liver, and was also expressed relative to blood radioactivity (tissue to blood ratios).

Results

Labeling of Platelets with Indium-Oxine

For the 25 experiments in which 111In-labeling was performed in an acidified plasma medium, the mean labeling efficiency was 72% ± 2.7% (SEM), and only 2.5% ± .25% (SEM) of 111In remained unbound to platelets in the surrounding medium in the re-injected labeled PRP. In the few experiments in which platelets were labeled in saline medium according to the technique of Thakur et al., labeling efficiencies were higher than 92%.

ADP-aggregation of 111In-labeled Platelets

No platelet aggregates were found by microscopic examination of PRP with phase contrast. Platelets drawn into ACD solution, labeled with 111In and resuspended in citrated plasma as described above, were found to be slightly more reactive than control platelets. Control platelets were harvested simultaneously from the same dog, but drawn into 3.8% sodium citrate rather than ACD solution and kept in suspension in citrated plasma (fig. 1). Complementary experiments revealed that platelets harvested from ACD-anticoagulated blood, kept in ACD-plasma and finally returned to citrated plasma, were slightly more reactive than platelets kept in citrated plasma for an equal time interval.
ADP 20µM ADP 10µM

Control PRP

FIGURE 1. ADP-induced aggregation of 111In-labeled platelets resuspended in citrated plasma after labeling. Control PRP was a sample of platelets collected from blood anticoagulated with a 3.8% sodium citrate solution and kept as PRP with that anticoagulant mixture. During the same venesection, another sample of platelets was isolated from blood drawn into ACD solution. These platelets were suspended in ACD solution during labeling with 111In. 111In-labeled platelets processed in ACD solution before being resuspended in 3.8% sodium citrate showed a little more reactivity to 10 µM and 20 µM ADP.

Elution, Sequestration and Blood-111In Radioactivity Curve

A. 111Indium Radioactivity in Plasma at the End of the Experiments

At the end of 12 experiments, blood samples were collected and the ratio of plasma radioactivity to whole blood radioactivity was calculated. Only 3.4% ± 0.45% (SEM) of 111In radioactivity was found in plasma, which suggested minimal elution or release of 111In from labeled platelets.

B. Organ Sequestration of 111In-labeled Platelets

The counting results for 111In (tagged to platelets) and 51Cr (tagged to red cells) radioactivities are presented in Table 1 for the lung, spleen, and liver. The results are expressed as tissue to blood ratios. The main result was a relatively large 111In spleen/blood ratio, 10 times higher than the 51Cr spleen/blood ratio, indicating that the spleen sequesters a fraction of the injected 111In-labeled platelets. Some trapping also occurred in the liver, but to a much smaller extent.

C. 111In-radioactivity Curve in Blood

Five to 10 min after the intravenous re-injection of 111In-labeled platelets and 51Cr-labeled red cells, the first arterial blood sample was taken, followed by serial samples every 30 min until the end of the experiment. The measured radioactivity in the first blood sample collected 5–10 min after re-injection of the labeled cells showed extreme variability between animals. Therefore, the second sample collected 30 min later was designated as the reference. The results from 6 animals presented in figure 2 are expressed as percent of radioactivity present in this reference sample. From this illustration, it is apparent that after 30 min of recirculation, 111In and 51Cr-blood radioactivity curves decrease slowly at a similar rate, which suggests an increase in blood volume, but no significant sequestration of labeled platelets.

Double Autoradiography with 111In-labeled Platelets and 14C-iodoantipyrine

A. Selective Elution of 14C-iodoantipyrine

Preliminary tests showed that methanol was a very efficient solvent for removing 14C-iodoantipyrine from brain tissue sections. A 3-min immersion in pure methanol was sufficient to elute the 14C-iodoantipyrine from the sections. Successive washings of several sections in the same bath led to a progressive contamination of the bath, which could ultimately imbue the tissue sections with a uniform redistribution of 14C if the bath was not changed.

B. Correlation Between Platelet Accumulation and Local Blood Flow

This correlation is illustrated in figure 3, which displays a typical set of autoradiographs obtained from two consecutive 40-µm brain sections treated as described in Materials and Methods. In this animal, extensive damage of one hemisphere of the brain was
produced with 110 μl of embolic air, and the 111In radioactivity was concentrated on the injured side (C). Accumulation of 111In was particularly dense in areas of gray matter with a very low flow (B) that corresponded to severely damaged foci of the brain (D: black area).

Discussion

To investigate the hypothesis that platelets accumulate in ischemically injured brains in dogs, two techniques allowing detection of platelets in tissue sections were tried in a model of focal brain ischemia: fluorescent microscopy with mepacrine-labeled platelets and autoradiographic detection of 3H-serotonin-labeled platelets. These two techniques were found to be relatively insensitive because they probe only a thin layer of brain on the surface of the tissue section and the brain’s vascular space is very small (a liberal estimate is 0.02–0.04 ml/g of wet tissue). These preliminary trials, however, were useful in suggesting that for sensitive detection of platelets in brain sections at a macroscopic level, thick tissue sections had to be explored to locate platelet aggregates scattered in the small cerebral vascular space, and the label tagged to platelets had to emit a sufficiently powerful radioactive signal to prevent internal absorption by the tissue section.

Following a review of various isotopes as potential platelet labels, 111In-oxine was selected because it offered the following advantages:

- Good labeling efficiency
- Preservation of platelet aggregability with minimal release of the label once it had been incorporated into the cell
- Suitability for double-label autoradiography with 14C and other long half-life radioisotopes due to its short half-life
- Emission of high-energy radiation to penetrate thick tissue sections and permit macroscopic autoradiography.

Discussion of the Technique

A. Animal Model

Two elements may trigger intravascular platelet aggregation in this mode: 1) direct effects of intravascular bubbles and 2) activation of the circulating platelets by damaged brain tissue.

B. 111Indium-labeling Efficiency

Because of the removal of plasma proteins, platelet labeling in a saline or buffer medium leads to very high

**Figure 4.** Brain to blood radioactivity ratios: tissue activity/tissue weight × 100

tissue activity/tissue weight

are displayed for 111In (tagged to platelets) and 51Cr (tagged to red cells). The injured side showed a major increase of the 111In tissue to blood ratio in the three zones (sections from anterior, middle and posterior tissue blocks), in contrast to the corresponding control ratios. The selected sample of maximally damaged tissue showed an 800% increase of the 111In tissue to blood ratio. None of these 111In ratio increases were associated with any significant variations of 51Cr ratios. Hatched bar = tissue to blood ratios of the injured hemisphere or of the maximally damaged area of the injured hemisphere. Clear bar = tissue to blood ratio of the corresponding part of the control hemisphere. PLA = platelet (111In tissue to blood ratio). RBC = red blood cells (51Cr tissue to blood ratio).
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... labeling efficiencies (higher than 90%), allowing the direct re-injection of labeled platelets along with the incubating medium. This eliminates additional centrifugation of the platelets. Several authors believe that ¹¹¹In labeling in saline or buffer does not harm platelets, 26 27 and that any activation of the platelets is reversible by simple reincubation in autologous plasma. 27 Other investigators, however, concluded that only labeling in autologous plasma preserves the platelet functions.28 29 Consequently, we chose to label platelets in a plasma medium, using the method of Scheffel et al 9-10 with minor modifications. A reasonable labeling efficiency was obtained in this manner (70%), which was higher than that reported by Scheffel et al 9 with human platelets and Dewanjee et al 29 with canine platelets.

C. Autoradiographical Techniques

To obtain autoradiographs depicting only ¹¹¹In distribution in sections containing both ¹⁴C and ¹¹¹In, experiments were conducted to find a treatment of brain sections that specifically removed ¹⁴C-iodoantipyrine, leaving ¹¹¹In-labeled platelets undisturbed. In addition to 2-2-dimethoxypropene originally suggested by Dr. L. Sokoloff and Dr. N.H. Diemer, pure methanol and aqueous solutions such as buffers and fixatives were also found suitable for this purpose. Among the eluents, methanol was selected because it facilitated fast drying of slides and excellent adhesion of tissue sections to the glass slides, and was compatible with eosin B staining of the white matter. The successive washings, as previously described, led to a complete elution of ¹⁴C and light pink staining of the white matter, without detectable removal of ¹¹¹In.

Behavior of Labeled Platelets

A. ¹¹¹In-labeled Platelet Aggregability

When ¹¹¹In-labeled platelets — drawn into the ACD solution and spun and labeled in ACD plasma — were resuspended in citrated plasma they were slightly more reactive to aggregation induced by ADP than control platelets collected simultaneously from the same dog, but anticoagulated with 3.8% sodium citrate (1:10) and suspended in citrated plasma during processing. Citrated PRP in the usual concentration contains just enough sodium citrate to prevent clotting, 15 but it does not allow platelet pelleting without aggregation. At 37°C incubation in sodium citrate, spontaneous aggregation can occur even without centrifugation. It has been reported that human platelets stored at room temperature in citrated plasma showed an initial increase in aggregability by ADP, followed by a gradual decline in sensitivity to that agonist. 30

B. Elution of ¹¹¹In from Platelets

Only 3.4% of the total ¹¹¹In radioactivity was found in plasma at the end of 12 experiments, indicating that only minimal elution and release of ¹¹¹In from labeled platelets had occurred. This confirmed the results obtained by Wilkinson 16 in which less than 2% of the radioactivity was eluted from canine platelets 1 hr after their re-injection. Similarly, Heaton et al 32 concluded that ¹¹¹In does not elute from platelets for at least 5 hr. Furthermore, ¹¹¹In is not released by platelets during the release reaction and aggregation. 33 34 One explanation of these data is that the ¹¹¹In ion binds strongly to large intracellular molecules; ¹¹¹In only cell lysis such as that produced by cytotoxic antibodies leads to a release of ¹¹¹In from cells, a release that is massive under these conditions. 34

The Possible Role of an Increase in Blood Volume to Explain an Apparent Accumulation of ¹¹¹In

A detectable increase of ¹¹¹In-labeled platelets in the brain may be due to platelet deposition, but it could also be due to a local increase in cerebral blood space. Increased capillary fragility could lead to focal extravasation of blood characterized by multiple, disseminated microhemorrhages. Gamma counting of brain samples from maximally affected brain regions in animals infused with both ¹¹¹In-labeled platelets and ⁵¹Cr-labeled erythrocytes suggested that an expanded blood space contributed very little to the apparent accumulation of platelets (fig. 4).

Conclusion

The new technique in which platelets are labeled with ¹¹¹Indium-oxine allows autoradiographic detection of the deposition of platelets in injured zones of brain tissue and correlation with local blood flow in the same area. The technique could be extended without difficulty to other organs. 35 ¹¹¹Indium-oxine can also be used to label leukocytes, 34 which suggests that autoradiographic detection of ¹¹¹In-leukocytes should also be possible. With respect to platelets, ¹¹¹Indium offers the fundamental advantage of minimal release of the label once it has been incorporated into the cell, in contrast to ¹⁴C-serotonin. Furthermore, it allows the simultaneous investigation of local blood flow by means of the ¹⁴C-iodoantipyrine technique. This principle of double autoradiography might also be extended to double-labeled platelets containing ¹¹¹Indium and ¹⁴C-serotonin, which would yield valuable information concerning deposition of platelets and platelet release reaction at the same tissue site.

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