Effect of Antiarrhythmic Drugs on In-111-Labeled Leukocytes: Chemotaxis and Adherence to Nylon Wool

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The influence of lidocaine (L) and procainamide (P) on the chemotactic ability and adherence to nylon wool of In-111-labeled human polymorphonuclear leukocytes (PMNs) was investigated. At the normal therapeutic levels of L (0.022 m*M* whole blood) or P (0.03 m*M* whole blood) no change in PMN function was observed. However, at and above five times the aforementioned blood levels of L, significant reduction in the chemotactic ability of PMNs was noted ($P < 0.005$). The adverse effects of In-111 radiation appeared insignificant at all L or P concentrations during the 3-hr observation period. The labeled PMNs were resistant to the toxic effects of a higher concentration of P than that of L, and the reduction in PMN chemotaxis and adherence to nylon wool was not apparent until the P concentration reached 1.5 m*M*.

J Nucl Med 23: 131–135, 1982

Functional integrity is of primary importance in the in vivo responsiveness of blood cells that have been isolated and labeled with a radionuclide in vitro before administration. Our previous studies have shown that the labeling procedure and the high radiation dose delivered by In-111 do not alter the chemotactic ability, antimicrobial capability, or structural integrity of In-111-labeled PMNs (*1*). During feasibility studies in which we used In-111-labeled autologous leukocytes to image the inflammatory response to acute myocardial infarction (MI) in 36 patients, 21 had positive images and 15 did not (*2*). Each of these patients had received, for different periods of time, antiarrhythmic drugs, L or P. In these patients, each milliliter of whole blood contained ~8 million PMNs on average, and the blood levels of the drugs were maintained at 0.022 m*M* for L (= 6 μg/ml whole blood) and 0.03 m*M* for P (= 8 μg/ml).

MacGregor et al. (*4*) have shown that L inhibits PMN adherence and prevents their delivery to inflammatory sites in rabbits. The effect is time and dose dependent. Furthermore, recent studies have indicated that HeLa cells, exposed to the local anesthetic P, show decreased resistance to low-LET irradiation, depending upon the drug concentration, duration of irradiation, and cell density (*5*). The above evidence suggests that L or P, combined with radiation from In-111, might affect the PMN function and, consequently, the ability of PMNs to accumulate in the area of infarction. We undertook this study to investigate the combined effects of L or P and In-111-induced radiation on the chemotactic and nylon adherence ability of PMNs.

MATERIALS AND METHODS

Isolation of PMNs. PMNs were separated from 30 ml of heparinized venous blood obtained from healthy human volunteers. Erythrocytes were eliminated by sedimentation, and leukocyte-rich plasma was centrifuged over a density gradient* for the removal of lymphocytes (*6*). Contaminating erythrocytes in the PMN pellet were then lysed and the PMNs washed and suspended in 5 ml of 0.9% NaCl. The viability of the PMNs was checked by Trypan blue dye exclusion, their con-

Received July 29, 1981; revision accepted Oct. 5, 1981.

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centration determined by a Coulter counter, and cell purity estimated by differential cell counting on a smear.

Control cells (C cells). Half a ml of the PMN suspension in 0.9% NaCl was withdrawn and a sufficient volume of sterile Gey's buffer† (containing 35% bovine serum albumin‡) was added to obtain 3×10^6 PMNs/ml, as required for the chemotaxis method of Zigmond and Hirsch (*7*). These cells were set aside and served as control cells.

Indium-111 control cells (C-In-111). The remainder of the PMNs were labeled with In-111 oxine‖ by the method of Thakur et al. (*8*), involving incubation at ambient temperature for 15 min. The radioactivity concentration in each experiment was maintained at approximately 5 μCi In-111 per million PMNs, so that each cell received a radiation dose (approximately 5800 rads) similar to that received by cells used in the in vivo investigations. Approximately half of the PMNs labeled in this manner were then suspended in a sufficient volume of Gey's buffer to obtain 3×10^6 cells/ml. These served as In-111-labeled control (C-In-111) cells.

Test cells. The remaining labeled cells were regarded as test cells and were suspended in cell-free plasma (approximately 8×10^6 PMNs/ml) and treated with L or P. The cell concentration and the use of autologous plasma closely mimicked the in vivo situation.

Experimental protocol. In separate sets of experiments, the test cells in plasma (8×10^6/ml) were incubated at 37 °C with the normal therapeutic levels of L (0.022 m*M*) or P (0.03 m*M*) for 0.5, 1, and 3 hr. In the next sets of experiments, the cells were incubated for 3 hr with L concentration increased to five and ten times the therapeutic levels, and the P concentration to five, ten, and 50 times the normal levels.

In other groups of experiments, designed to imitate a situation in which patients were receiving L or P before having blood drawn for cell labeling, the test cells were preincubated with L or P for 2 hr, then labeled with In-111 and incubated again with L or P for 1 hr. Each set of experiments was repeated three to ten times. These cells were then centrifuged to eliminate plasma and suspended in Gey's buffer to obtain 3×10^6 PMNs/ml. Following these procedures, the C, C-In-111, and test cells were studied for chemotaxis and adherence to nylon wool.

Chemotaxis. The chemotaxis assays were performed in modified Boyden chambers using the method of Zigmond and Hirsch (*7*). A cellulose nitrate filter,§ 1.2 μm, 13 mm diameter, placed in the chamber separated the chemotactic agent [5 mg/ml Na caseinate in phosphate buffer pH 7.2 (*9*)] from a 0.5-ml cell suspension in Gey's buffer. In each set of experiments 12 chambers were used: two for C cells without chemotactic agent, and two for C cells and four for each of the C-In-111 and test cells with chemotactic agent. The chambers were then incubated at 37 °C for 90 min, filters were withdrawn and stained, and the distance between the filter top and leading cell front in the filter was measured with an optical micrometer. The cell migration in five separate fields was measured on each filter and the average distance recorded. The distance traveled by C cells without the chemotactic agent demonstrated spontaneous cell migration and served as internal control for reagent sterility and any variation in experimental conditions. Any experiment in which the spontaneous cell migration was greater than 35% of that of the control cells with chemotactic agent was discarded.

In each set of experiments, the average migration of C and C-In-111 cells was regarded as 100%. The average migration of test cells relative to each of the two controls was then calculated. The corresponding numbers obtained from each of the three to ten sets of experiments for each concentration of L and P were used to calculate the grand mean (± s.e.). These were then statistically compared (Student's *t*-test) with grand means similarly obtained for the other concentrations of L and P.

Test of adherence to nylon wool. Nylon wool columns,¶ ~4 × 0.3 cm, were prepared in Pasteur pipettes, and 0.5 ml of C-111-In and test PMN suspension were loaded onto each of two separate columns (*10*). Each column was then washed with 2 ml 0.9% NaCl and the eluate collected. The radioactivities eluted and remaining on the column were measured in order to calculate the percentage of radioactivity retained on the column. The results were processed in a manner similar to those in the chemotaxis test.

RESULTS

On the average (94 ± 4)% isolated cells were PMNs, of which (93 ± 3)% excluded Trypan blue dye. The migrations of both types of control cells (C and C-In-111) were invariably equal (not different statistically) in each set of experiments. The incubation of PMNs with therapeutic concentration of L for 0.5, 1, or 3 hr neither affected chemotactic ability nor changed the nylon-wool adherence of labeled PMNs (Table 1). However, incubation of labeled PMNs for 3 hr with five times the therapeutic levels of L (0.11 m*M*), significantly reduced the PMN chemotaxis ($P < 0.005$). The results (Table 2) indicated that incubating labeled PMNs in 0.11 m*M* L for 3 hr—or preincubating unlabeled PMNs for 2 hr, labeling them with In-111, and incubating them again for 1 hr in 0.11 m*M* L—reduced the cells' chemotaxis as well as the nylon-wool adherence. However, there was no statistical difference between the degree of reduction in the two groups. When the L concentration was increased to ten times the normal therapeutic level, there was further reduction in chemotaxis and nylon-wool adherence of labeled PMNs (Table 2).

Labeled PMNs exposed for 3 hr to up to 10 times the

TABLE 1. EFFECT OF LIDOCAINE AT THERAPEUTIC AND HIGHER CONCENTRATIONS AS A FUNCTION OF INCUBATION TIME*

L conc., with PMNs 7–8 X 10^6 per ml	Period of incubation (hr)	% Chemotaxis w.r.t. C	% Chemotaxis w.r.t. C-In-111	% Adherence w.r.t. C-In-111
Th (0.022 m*M*)	0.5	—	119.9 ± 27.3	94.6 ± 32.6
(0.022 m*M*)	1	100.7 ± 10.1 NS	104.7 ± 8.2 NS	87.7 ± 14.3 NS
(0.022 m*M*)	3	110 ± 8.7 NS	103.5 ± 13.7 NS	99.2 ± 16.6 NS
5 X Th (0.11 m*M*)	3	82.1 ± 6.2 ($p < 0.005$)	79.4 ± 4.8 ($p < 0.005$)	82.2 ± 25 NS

* Key to symbols used: C: control cells (unlabeled); C-In-111: control cells labeled with In-111; Th: average therapeutic blood levels; L: lidocaine; P: procainamide; NS: statistically insignificant difference ($P > 0.025$); w.r.t.: with respect to.

therapeutic concentration of P—or unlabeled PMNs exposed to the same concentration of P for 2 hr and for another hour after they had been labeled—demonstrated no significant change of their function (Table 3). However, when labeled cells were incubated for 3 hr with 50 times the therapeutic level of P, the PMN chemotactic ability was significantly reduced ($P < 0.005$) and the nylon-wool adherence was decreased (Table 3).

DISCUSSION

The cationic antiarrhythmic drugs, when in higher than therapeutic concentrations, have been shown to inhibit a number of granulocyte functions in vitro (*4*). Our interest in these studies was generated from the use of In-111-labeled PMNs to image inflammatory response to acute MI in patients receiving L or P. Our aim was to evaluate the combined effects of the exposure to

TABLE 2. EFFECT OF INCREASING LIDOCAINE CONCENTRATION (2 HR PREINCUBATE, LABEL WITH In-111, 1 HR POSTINCUBATE)*

L conc., with PMNs 7–8 X 10^6 per ml	Period of incubation	% Chemotaxis w.r.t. C	% Chemotaxis w.r.t. C-In-111	% Adherence w.r.t. C-In-111
Th† (0.022 m*M*)	3 hr post	110 ± 8.7	103.5 ± 13.7	99.2 ± 16.6
5 X Th† (0.11 m*M*)	2 hr pre 1 hr post	88.7 ± 8.7 ($P < 0.005$)	91 ± 7.1 ($P < 0.025$)	86.8 ± 3.8 NS
10 X Th† (0.22 m*M*)	2 hr pre 1 hr post	78.2 ± 10 ($P < 0.005$)	88.4 ± 9.2 ($P < 0.01$)	76.5 ± 10 NS
5 X Th‡ (0.11 m*M*)	3 hr post	82.1 ± 6.2	79.7 ± 4.8	82.2 ± 25
5 X Th‡ (0.11 m*M*)	2 hr pre 1 hr post	88.7 ± 8.7 NS	91 ± 7.1 NS	86.8 ± 3.8 NS

* See Table 1 for key to symbols used.

† These data indicate that at 5 and 10 times the concentrations of L, the PMNs' chemotaxis was significantly decreased and adherence was reduced.

‡ These data suggest that incubating PMNs with same L concentrations for 3 hr after labeling, or 2 hr before labeling followed by 1 hr after labeling, reduce PMN functions to a similar degree.

TABLE 3. EFFECT OF INCREASING PROCAINAMIDE CONCENTRATION AND OF PRE- AND POST-INCUBATION

P conc. with PMNs 7–8 × 10^6	Period of incubation	% Chemotaxis w.r.t.-C	% Chemotaxis w.r.t. C-In-111	% Adherence w.r.t. C-In-111
Th† (0.03 m*M*)	3 hr post	92.9 ± 24	92.8 ± 10	88.2 ± 8.6
5 × Th† (0.15 m*M*)	3 hr post	87.5 ± 8.7 NS	96.6 ± 7.6 NS	82.4 ± 9 NS
50 × Th† (1.5 m*M*)	3 hr post	66.2 ± 17.4 P < 0.005	71.1 ± 12.5 P < 0.005	81.5 ± 7.5 NS
Th‡ (0.03 m*M*)	3 hr post	92.9 ± 10	92.8 ± 10	88.2 ± 8.6
5 × Th‡ (0.15 m*M*)	2 hr pre 1 hr post	91.9 ± 9.5 NS	105.4 ± 4.6 NS	81 ± 5 NS
10 × Th‡ (0.3 m*M*)	2 hr pre 1 hr post	105 ± 14 NS	105 ± 21 NS	79.6 ± 7.2 NS

* See Table 1 for key to symbols used.

† Data indicate that, unlike 5 × Th L concentration, 5 × Th P concentration was not toxic to In-111-labeled PMNs. At 50 × Th P concentration, however, the PMN chemotaxis was significantly reduced and adherence was decreased.

‡ Analogous to L data (Table 2), these data also suggest that incubating PMNs with P for 3 hr after labeling, or for 2 hr before labeling followed by 1 hr after labeling, has no significantly different effects on PMN functions.

therapeutic levels of L or P and the In-111-delivered radiation on In-111-labeled human PMNs. We chose chemotaxis and adherence to nylon wool as the in vitro functional tests. The ideal way to perform these investigations would have been to study the functions of PMNs separated from MI patients given In-111 PMNs. However, since there is no way to separate In-111-labeled PMNs selectively from the PMN population in whole blood, we chose to incubate In-111 PMNs with L or P in vitro. The maximum incubation time was limited to 3 hr, since the control cells could not be preserved at full viability in Gey's buffer, at 37 °C, for longer than this period. Under these conditions, our results indicated that at the normal therapeutic blood levels of L, the chemotactic function and the adherence of In-111 PMNs remained unaltered. The chemotactic ability of labeled PMNs, however, was significantly reduced ($P < 0.005$) when the L concentration was increased fivefold or tenfold. The results also indicated that incubating PMNs with five or ten times the therapeutic L concentration before the labeling of PMNs with In-111, or incubating them for 2 hr before labeling and 1 hr after labeling, reduced their chemotactic ability to a similar degree. This suggested that the PMN exposure to a high concentration of L alone adversely affected the chemotactic ability of PMNs, and that the short period of radiation exposure was not a significant contributing factor. This conclusion is also supported by the fact that the migration of C-In-111 cells was never significantly less than that of C cells in any experimental group.

The results of the PMN exposure to P were somewhat different from those with L in that the exposure to up to ten times the therapeutic levels of P had no significant influence on the PMN functions.

The exposure of PMNs to local anesthetics or antiarrhythmic drugs causes structural changes and thereby reduces cell pseudopod formation (*4*). Our results indicated that this phenomenon does occur but only at levels of L or P higher than those normally specified. These observations concur with those of MacGregor et al. (*4*), who reported reversible granulocyte adherence upon exposure to therapeutic levels of L, and observed reduced PMN adherence after large bolus dose followed by an infusion of L in rabbits.

The influence of In-111-delivered radiation during the 3-hr period upon the functional integrity of PMNs appeared insignificant. This is in agreement with our previous findings (*1*). This observation, however, does not rule out the possibility that the radiation received by PMNs for longer than 3 hr may add to the inhibition of cell function. Such effects, inhibiting the colony-forming ability of HeLa cells, have been reported by Djorjevic

(5). The previously unreported observations that labeled PMNs were resistant to the toxic effects of much higher concentration of P than those of L are worthy of further investigation.

FOOTNOTES

* Pharmacia, Ficoll-Hypaque.
† GIBCO.
‡ Sigma.
‖ Medi + Physics.
§ Millipore, Inc.
¶ 250 mg, Fenwall.

ACKNOWLEDGMENTS

The work was supported by DOE contract No. EY-76-S-02-4078 and by AHA grant No. 78-957.

The helpful advice of Dr. H. L. Malech and the skillful typing of Alyce Harrity are gratefully acknowledged.

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