Leukokinetics in Peripheral Blood (II) Measurement of Peripheral Leukocytes Radioactivity Labeled with Tritiated Thymidine ($^3$H-HdR) or Diisopropylfluorophosphate (DF$^{32}$) in Vivo

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The radioactivity of the peripheral leukocytes labeled with $^3$H-TdR or DF $^{32}$P in vivo was measured in leukemic and normal individuals. Tritiated thymidine (5 mCi) or DF $^{32}$P (150-200 µCi) was injected intravenously in the patients with chronic myelocytic leukemia (CML), acute leukemia and gastric cancer who was a limited life expectancy and inoperable.

Peripheral blood samples were drawn at predetermined intervals and leukocytes were isolated by the method of sedimentation with dextran and lysis by saponin. The specific activity of the leukocytes were measured and mg of leukocyte nitrogen in each sample was determined. Radioactivity expressed as CPM/mg leukocyte nitrogen.

The measurement of $^3$H-labeled leukocyte specific activity was as follows. The leukocytes were washed twice with isotonic saline. They were dissolved in 5% sodium hydroxide (at 60°C, 15 minutes) and 0.1 ml of the resulting solution was added to 18 ml of a scintillation mixture, 70% toluene, 30% methanol containing 5g of PPO and 0.05 g of POPOP per L. The samples were counted in a “Packard Tri-carb Liquid Scitillation Spectrometer”. Quenching was negligible with the use of external standardization.

The urinary excretion of $^3$H-TdR was rapid within a day. The disappearance of radioactivity from plasma corresponded with rapid urinary excretion. The result supported that $^3$H-TdR was used as a flash label without re-utilization.

The label of $^3$H-radioactivity in a normal individual remained low in the peripheral blood until the fourth day, but sharply increased, reaching peak labels at about a week. After two weeks, the curve reached base line. The curve of DF $^{32}$P-radioactivity in four normal individuals could be divided into three phases. At 1st phase, there was rapid decrease in leukocyte radioactivity. (T1/2 6-10 hours) Then, leukocyte radioactivity remained constant until the 8th-10th day. (2nd phase) At 3rd phase, there was a final decrease in an exponential fashion. The time course of labeled percents of granulocytes in radioautography well corresponded with that of $^3$H-radioactivity of the peripheral leukocytes. From these data, it was suggested that granulocytes were reseaved in bone marrow for about four days after their proliferation and maturation, then they appeared in the peripheral blood, where influx and exflux of the granulocytes remained equilirium. The median sojourn time in peripheral blood was about 14 days. It was considered that the granulocyte kinetics in normal man was quite regular.

Leukokinetic curve of CML patient labeled with $^3$H-TdR was characterized by an initial high level or radioactivity followed by a peak at 36 hours and the 7th day. Disappearance curve of DF $^{32}$P radioactivity from two CML patients indicated random destruction.

In two cases of acute leukemia $^3$H-labeled leukocytes had high radioactivity initially, reached a maximum at the 3rd day. Analysis of radioautography from these patients failed to reveal the same type of the curve.

Liquid scitillation counting of peripheral leukocytes might be a useful tool in the studies of leukokinetics in normal man, proliferation and maturation of leukemic cells and therapy of the leukemia, especially by using the radioautography at the same time.