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INVESTIGATIONS INTO AGENTS FOR IMPROVING CELL LABELING WITH
POSITRON AND GAMMA-EMITTING RADIONUCLIDES

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Slide 1: The purpose of this study was to investigate the preparation of radiopharmaceuticals and the ability to label W.B.C. with a longer lived B^+ emitting radionuclide than Ga-68 and to be able to label platelets in plasma efficiently with a convenient radionuclide.

Cellular blood elements labeled with In-111-oxine have found many experimental and clinical applications by gamma camera imaging. Accurate quantitation of kinetics of blood cell infiltration in lesions such as myocardial infarctions of transplanted organs with or without therapeutic interventions could be made feasible with emission tomography

Slide 2: Ga-68 is a B^+ emitter, 88% of the time, its $t_{1/2}$ is a little over one hour which is too short compared to the time white blood cells remain in circulation. Co-55 is also a B^+ emitter 81% of the time but its $t_{1/2}$ is 18.5 hrs. which makes it better suited for labeling W.B.C. for certain applications.

In our investigations we used Co-58 which has a convenient $t_{1/2}$ of 71.3 days to study the feasibility of labeling W.B.C. with either Co-oxine valence II or III.

Slide 3: Co-oxine in the valence III was prepared by treating Co with H_2O_2 then the solution was dried under N_2 , dissolved in acetate buffer and then the synthesis of Co-oxine was carried out in the following manner:

Slide 4: Co-58 and 100 ug oxine were placed in each of a series of 1ml acetate buffer solutions of different pH's. Cobalt oxine was formed immediately at room temperature. The amount of radioactivity extracted into chloroform was an indication of the complex formation.

Slide 5: The effect of pH on the extraction of Co-oxine is shown. Between 88 and 95% of Co-58 was extractable into chloroform at a pH range of 5.4-8.2 .

Slide 6: For cell labeling, leukocytes were harvested by gravity sedimentation and centrifugation.

Slide 7: The % uptake of radioactivity by W.B.C. is shown. In each test tube, about 130 million cells per ml of saline incubated with tracer amounts of Co-58-oxine of valence II or III for 20 minutes at room temperature. Both Co-oxinates valence II & III labeled the cells respectively with 22 and 29.6% of the added activity. However, only 14 and 15% of the originally added activity remained with the cells after washing.

The stability constant of Co-oxine, $\log k$, is 9.6 and that of In-oxine is 11.8 which are comparable. While In is retained in the cells Co is not. The lower retention of cobalt may be attributed to its lower selectivity by cytoplasmic components.

For labeling platelets in plasma, Ru-97 was selected in this study for the following reasons:

Slide 8: For imaging purposes some of the physical characteristics of Ru-97 are similar to those of In-111. Both have comparable half lives, about 3 days; they decay by E.C.; Ru-97 has a major gamma energy of 215 keV (91%) while In-111 has two major ones.

In our investigations Ru-103 was used due to its convenient 39.6 days $t_{1/2}$. Unlike In-111, Ru was not expected to bind blood protein. To confirm this (slide 9), In-111-oxine was incubated with plasma for 30 minutes at ambient temperature and then spotted on an ITLC and developed in 85% methanol. 100% of the radioactivity stayed at the origin. When In-oxine was spotted directly on ITLC and developed in the same solvent, 81% of the radioactivity had an R_f value of 0.6. When the same treatment was done on Ru, in both cases Ru traveled with the solvent front indicating that it did not bind to plasma proteins. This property was also confirmed by electrophoresis and autoradiography.

Slide 10: When we compare the absorbed doses of In-111, Tc-99m and Ru-97 at the cellular level, that is considering the equilibrium absorbed dose constants of nonpenetrating radiations as a comparative measure, and assuming similar subcellular localization, the radiation dose to the cell from Ru could be as little as 1/3 that of In-111. If we compare the average doses to the spleen per mCi of homogeneously distributed radioactivity with an effective $t_{1/2}$ equal to physical $t_{1/2}$, we expect Ru to deliver half the dose of In-111. Tc-99m delivers the least dose but at the moment labeling with Tc is not so efficient.

Bearing in mind these advantages we tried to evaluate ^{3}Ru complexes in labeling platelets in plasma.

Slide 11: For this we isolated human platelets in ACD by differential centrifugation and concentrating a known number of platelets in plasma.

Slide 12: The complexes were Ru-oxine, Ru-oxine-7-carboxylic acid and Ru, 1, 2, 3, 4-tetrahydrooxine-7-carboxylic acid. Ru-oxine, the most lipophilic complex was incorporated most into the cells while as the lipophilicity decreased, the percent of radioactivity incorporated by the cells was reduced. However a large proportion of Ru was retained even after washing the cells twice.

It was obvious then that Ru-oxine was the compound to investigate further. Two related methods were used in the preparation of Ru-oxine.

Slide 13: Method A, was done in our lab and was similar to the preparation of Co-oxine and In-oxine. Except that for Ru-oxine, pH, temperature and the duration of refluxing of the reactants was an interaction that influenced the completion of the synthesis in the following manner:

Slide 14: When the refluxing time was for one hour at 85°C , between 84-95% of the radioactivity was extractable into chloroform between pH's 4-8 respectively.

Slide 15: When pH was held constant at 7 and time of refluxing the reactants was varied at 90°c and 50°c about 40 minutes of refluxing at 90°c was necessary for the completion of the synthesis reaction.

Slide 16: Method B was done at Brockhaven where RuCl_3 and oxine in ethanol were placed in saline, the pH was adjusted to between 6 and 7 then refluxed in boiling water for 60 minutes in a sealed vial. The resulting solution was then ready for cell labeling. Radiochemical purity, when checked with TLC was about 83%. Using Ru-oxine prepared by this method several parameters that affect platelet labeling were investigated.

Slide 17: 1. The effect of platelet concentration. When the concentration varied between 9.3×10^7 to 20 times that amount, the percent yield increased linearly from 10 to 50% with a reliability factor of 94%.

2. The effect of citrate. In this study platelets were isolated, washed and suspended in saline to which an increased concentration of ACD was added. 3.51mg citrate/ml is what would be present in plasma at the time of collecting the blood. Therefore going 2 pts. above and below this concentration and no ACD; we observe a 40% difference between when there was no ACD and when there was 14.0mg citrate/ml.

Slide 19: 3. The influence of incubation time on labeling platelets in plasma indicated that a maximum yield of about 55% was obtained between 45 and 60 minutes incubation at ambient temperature.

SUMMARY

It was possible to label leukocytes with Co-oxine, but a large proportion of the radioactivity was eluted from the cells upon washing. Ruthenium oxine labeled platelets efficiently in plasma while negligible proportion of radioactivity was eluted from the cells.

Three factors influence the labeling efficiency of the cells.

1. duration of the incubation period
2. cell concentration
3. and ACD conc.

FUTURE WORK

In this feasibility study, a limited radiochemical purity analysis was done. Instead we assumed that we are dealing with one species of Ru-oxine. The chemical composition of Ru-oxine will be determined and the in vivo evaluation of labeled platelets will be studied.