The Effect of Diisopropylfluorophosphonate (DFP) on Platelet Survival in the Rabbit

By Yi-Han Chang

One of the methods used for the determination of platelet and erythrocyte lifespan is the in vivo labeling of the cells with radioactive diisopropylfluorophosphonate (DFP[^2]), first reported by Cohen and Waringa in 1954[^1], and based on the irreversible combination of DFP[^2] with esterases present in the cell membrane. The normal range of platelet survival in humans determined by this method is 8 to 14 days[^2,3]. Platelet survival time when determined by this method is decreased in atherosclerosis[^4], thrombocytopenia[^5,6] and primary gout[^7], and is prolonged in subjects with thrombocytosis[^8]. In recent years, DFP[^2] has been used extensively in the studies of drug effect on platelet survival in humans and rabbits. Prolongation of survival of human and rabbit platelets by sulfinpyrazone and of human platelets by dicumarol and heparin has been reported[^8,11], but it has never been established whether or not DFP[^2] itself influences platelet survival. The present study was undertaken to answer this question.

Materials and Methods

**Administration of DFP[^2]**

For the in vivo labeling of rabbit platelets, a solution of DFP[^2] in sterile propylene glycol (specific activity 235 µc./mg., 0.85 mg./ml.; Nuclear Chicago Corp.) was injected slowly into a marginal ear vein. Blood samples (4 ml.) were collected from the marginal ear vein of the opposite ear in the morning on the day after injection and daily thereafter for 5 days.

**Isolation of Platelets**

The procedure of Mustard[^12], which was based on the method of Leeksma and Cohen[^13], was employed with minor modifications. All procedures were carried out at room temperature. Blood (4.0 ml.) was added to 1.0 ml. of a disodium ethylenediaminetetraacetate (EDTA) solution (2 per cent EDTA in 0.85 per cent saline) in a silicone-coated glass centrifuge tube. The blood sample was centrifuged at 600 r.p.m. (RCF 80) for 15 minutes in an International Centrifuge (Model V). The supernatant platelet-rich plasma was transferred to a silicone-coated glass centrifuge tube. The volume of the platelet-rich plasma was measured and the platelets counted by microscopy or by means of a Coulter counter. The plasma was then centrifuged at 2100 r.p.m. (RCF 940) for 10 minutes. The supernatant was discarded and the platelet button gently transferred to a silicone-coated glass centrifuge tube and suspended in 2.0 ml. of a saline-EDTA solution for washing (one part of the above EDTA solution, nine parts 0.85 per cent saline). The platelet suspension was centrifuged at 1900 r.p.m. (RCF 760) for 10 minutes. The supernatant was discarded and the platelet button suspended in 0.5 ml. water. This platelet suspension

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Fig. 1.—Semilog plots of the regression curves obtained by a least squares fitting of the observations of per cent platelet survival in five groups of rabbits with in vivo DFP platelet tag (I—0.3 mg./Kg.; and V—0.46 mg./Kg.). Each point represents the mean of six values. The number next to each point is the standard error for that mean.
was then transferred to a stainless-steel planchette and the material air-dried at room temperature overnight and subsequently placed in a desiccator under vacuum for 24 hours. The dry weight of the sample was then determined.

Measurement of Radioactivity

Samples were assayed by liquid scintillation counting technics in a Nuclear Chicago liquid scintillation spectrometer (Model 725). The dried platelet samples were dissolved in 3.0 ml. of a Hyamine solution (10-X, Rohm & Haas), to which was then added 15 ml. of a toluene scintillator solution containing 0.6 per cent diphenloxazole (PPO) and 0.02 per cent p-bis-2-(5-phenyloxazolyl)-benzene (POPOP) followed by 0.2 ml. glacial acetic acid. All samples were counted on the same day to eliminate the need to correct for radioactive decay. Each sample was counted for 20 minutes or to accumulate a minimum of 2000 counts. The radioactivity was corrected for the background radiation and expressed as counts per minute \(10^9\) platelets or per mg. platelet dry weight. In our experiments, the fall-off of the platelet-bound radioactivity appears to conform to an exponential decay, and our results have been computed accordingly by fitting a regression line to the logarithms of the activities by the method of least squares.

RESULTS AND DISCUSSION

Figure 1 shows the platelet survival curves obtained by a least squares fitting of the observations of per cent platelet survival in five groups of rabbits (six in each group, receiving 0.10, 0.15, 0.20, 0.30 or 0.46 mg./Kg. DFP\(^{32}\)). plotted on a semilog scale. Each point represents the mean of six values. The number next to each point is the standard error for that mean. The half-life values of the survival curves are 1.55, 1.73, 2.00, 2.20 and 4.67 days, respectively. The relationship between the platelet half-life values and the doses of DFP\(^{32}\) administered is shown in Figure 2. A gradual increase in platelet half-life with increasing amounts of DFP\(^{32}\) administered is apparent.

DFP attaches to various proteins, during which process it changes to diisopropylphosphate (DIP).\(^1\) Although its major attachment has generally been considered to be to the cholinesterase molecule, DFP will attach to any protein that has a serine group.\(^1\)\(^4\) Indirect evidence was reported recently suggesting that DFP causes "injury" to platelets.\(^1\)\(^5\) It also inhibits platelet release of nucleotides induced by thrombin, collagen, antigen-antibody complexes and gamma-globulin coated polystyrene.\(^1\)\(^6\) The mechanism whereby DFP brings about a prolongation of platelet survival is not known. It may interfere with the functions of platelets, such as the maintenance of capillary integrity or phagocytosis, in the normal performance of which they are removed from the circulation, or it could affect the basic mechanism through which platelets are removed by the spleen.

Due to the relatively low specific activity obtainable in the preparation of DFP\(^{32}\) and the short half-life of P\(^{32}\) (14.3 days), it is customary to employ a constant amount of radioactivity rather than constant amount of DFP in the labeling of platelets with DFP\(^{32}\) for survival studies. The amount of DFP administered could thus vary, depending on the specific activity of the sample used. This could result in significant variations in the survival values determined.

The amount of DFP\(^{32}\) used in human studies is usually kept below 0.035 mg./Kg.\(^1\)\(^3\)\(^9\) At this dose level, it may not affect platelet survival significantly.
Fig. 2.—The relationship between the platelet half-life values and the doses of DFP\textsuperscript{122} administered. Each point represents the mean platelet half-life of six animals. The number next to each point is the standard error for that mean.

Nevertheless, the finding reported here points out the importance of standardizing the amount of DFP\textsuperscript{122} administered in comparative experiments, especially those carried out in experimental animals where the amount of DFP\textsuperscript{122} injected is substantial.

**Summary**

Diisopropylfluorophosphonate was found to influence platelet survival. The possible mechanisms of action and the importance of standardizing the amount of DFP\textsuperscript{122} administered in platelet survival experiments are discussed.

**SUMMARIO IN INTERLINGUA**

Esseva trovate que diisopropylfluorophosphonato exercet un influentia super le superviventia plachettal. Le mechanismos possibile del action e le importantia de standardisar le
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quantitate del diisopropylfluorophosphonato a P32 administrate in experimentos concernite con le superviventa de placchetas es commentate.

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REFERENCES

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