Rapid Equilibration of Warfarin Between Rat Tissue and Plasma

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Plasma and tissue concentrations of warfarin in the rat were measured as a function of time following a 10 mg/kg intravenous dose. The mathematical interpretation of the experimental results suggested that the data could be explained in terms of a two-compartment open model. Following equilibration, which occurred within a few minutes after injection, individual tissue levels and plasma levels of warfarin were found to be always directly proportional.

KEY WORDS: warfarin; linear pharmacokinetics; tissue binding; plasma protein binding; tissue distribution.

INTRODUCTION

The purpose of this study was to derive a pharmacokinetic model which would describe the relationship between tissue and plasma concentrations of warfarin in rats, as a function of time, following an intravenous dose of sodium warfarin, 10 mg/kg.

Forty male Sprague-Dawley rats with an average weight of 300 g were used in the study of eight time intervals: 1, 5, 15, 30, 60, 120, 180, and 240 min. Five animals were used for each time interval, four serving as recipients and the fifth as a blank.

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EXPERIMENTAL

Animal Treatment

The rats were starved the night before the experiment and during the experiment; water only was allowed *ad libitum*. The rats were weighed in the morning prior to the experiment, and 3 mg of sodium warfarin was administered intravenously by tail vein, based on their average weight. The rats were not anesthetized during the injection.

At the end of each time interval, the corresponding rats were anesthetized with ether U.S.P. An abdominal incision was made, and an immediate blood sample was taken from the bifurcation of the inferior vena cava. The needle was then detached and the sample transferred slowly to prevent hemolysis against the inside of a 17- by 100-mm plastic vial containing 0.2 ml of sodium citrate, 25% w/v. The plasma was separated by centrifugation and transferred by pipette into new plastic vials, capped, and frozen at $-4^\circ$C. The rats were then decapitated, and the following were organs excised, transferred to plastic vials, capped, and frozen at $-4^\circ$C: brain, heart, lung, spleen, and liver.

Tissue homogenates were prepared and then refrozen until assayed. Each homogenate run represented the same organ for a given time interval. For example, all five hearts excised at 1 min after injection were first thawed, blotted, and weighed, and then exactly 0.7 g of tissue from each heart was weighed into a separate, tared 15-ml Broeck glass tissue grinder and homogenized with 7 ml of phosphate buffer, pH 7.25 (0.1 M). The homogenate was then transferred to glass tubes, stoppered, and frozen at $-4^\circ$C until time of assay.

Assay Methodology

The amount of warfarin in plasma and tissues at various time intervals was determined by the modified O'Reilly method with a few modifications (1). The additional changes involved the extraction procedure, elimination of separatory funnels, and use of pyrex glass wool for filtering purposes. The final method is given in detail below.

Blank rat plasma and tissue samples freshly frozen at $-4^\circ$C, followed by thawing, homogenizing with warfarin as a spike, and refreezing at the end of 4 months, and finally thawing again at intervals over the next 5 months, yielded excellent Beer's law plots. O'Reilly also reported that results of warfarin determinations on plasma, urine, and stool stored in the frozen state for several months, even when the specimens were repeatedly thawed and frozen, were not significantly different from those obtained on fresh samples (2). It was also determined that human plasma, O-positive and not