Efficacy of Water-Soluble Derivatives of Vitamin K₁ in Counteracting Drug-Induced Hypoprothrombinemia

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Hypoprothrombinemia, resulting from simple vitamin K lack such as occurs in dietary deficiency in the chick or in absorptive difficulties in man, responds almost equally well to treatment with vitamin K₁ (2-methyl-3-phytyl-1,4-naphthoquinone) or Menadione (2-methyl-1,4-naphthoquinone) and water-soluble Menadione analogues.¹³ That this may be true also in hypoprothrombinemia of the newborn is suggested by available data.³⁵ There is a striking difference in activity between vitamin K₁ and Menadione, however, in reversing hypoprothrombinemia induced in mammals by drugs like Sulfadoxine, Dicumarol [3,3'-methylenebis (4-hydroxycoumarin)] and its analogues and phenylindanedione and analogues such as Dipaxin (2-diphenylacetyl-1,3-indanedione). Some investigators have suggested that Menadione and its water-soluble derivatives may be essentially devoid of activity in certain drug-induced hypoprothrombinemias.⁶⁹ Early studies in this laboratory showed vitamin K₁ to be considerably more effective than Menadione in preventing prothrombinopenia due to Sulfadoxine in rats and dogs¹ or that due to Dicumarol in these same species.¹¹ Subsequent studies by others in animals as well as in man have confirmed the superiority of vitamin K₁ and K₁ oxide over Menadione and its analogues in correcting hypoprothrombinemia resulting from oral anticoagulants.²¹ The physical, chemical and biologic properties of the K vitamins are described in a recent review article.²¹

Up to the present time, vitamin K₁, which is an oil, has been employed intravenously in emulsion form. It seemed desirable, therefore, to determine whether water-soluble derivatives of vitamin K₁ would be as effective as the emulsion in counteracting drug-induced hypoprothrombinemia.

The current investigation deals principally with the comparative efficacies of vitamin K₁ emulsion and the water-soluble disodium salt of 2-methyl-3-phytyl-1,4-naphthohydroquinone-1,4-diphosphate, administered by the intravenous, oral and intramuscular routes in dogs rendered hypoprothrombinemic by Dicumarol. Limited work is included also on another anticoagulant, Dipaxin, and on several other water-soluble derivatives of vitamin K₁. A preliminary account of this work has been reported.²²

Materials and Methods

A group of 25 healthy, adult beagle dogs of both sexes were employed for these studies. They were housed in air-conditioned quarters, maintained at a temperature of about 75°F and relative humidity of 40–60%. A nutritionally adequate stock ration was provided fresh daily and water was given ad libitum. Prothrombin times were determined on citrated plasma by the one-stage method, essentially according to Quick.³⁹ The dogs were used...
repeatedly in this study but were permitted a minimum of one week's rest between experiments.

In curative-type experiments, anticoagulant was given orally on each of two consecutive days and then a single dose of the vitamin K compounds was administered two, or occasionally three, days after the second anticoagulant dose. The course of developing hypoprothrombinemia served as the basis for subdividing the animals into comparable experimental groups. Groups of three dogs each received a test K compound and two or three dogs received none. Every experiment, therefore, was complete in itself, inasmuch as non-vitamin K-treated controls were always included. Prothrombin times were determined once daily prior to vitamin K treatment. On the day that the K compounds were administered, determinations were made just before, and 1, 2½ and 5 hours after treatment. In prophylactic experiments, anticoagulant and vitamin K compounds were given on the same days.

The anticoagulants, Dicumarol and Dipaxin, were suspended in 2% methyl cellulose solution and administered by stomach tube. In all instances the water-soluble vitamin K compounds were compared with vitamin K\textsubscript{1} on a molar basis. Accordingly, a milligram of vitamin K\textsubscript{1} was compared with the following amounts of the other compounds tested: 1.46 mg. of 2-methyl-3-phytyl-1,4-naphthohydroquinone-1,4-diphosphate disodium salt; 1.23 mg. of the 4-monophosphate monosodium salt; 1.35 mg. of the 1-propionate-4-phosphate monosodium salt. Concentrations of the vitamin K solutions were adjusted so that equal volumes were given on a body weight basis.

The formula of the water-soluble diphosphate derivative of vitamin K\textsubscript{1} follows:

\[
2\text{-Methyl-3-Phytyl-1,4-Naphthohydroquinone-1,4-Diphosphate}
\]

\[
\text{HO-}P-OH
\]

\[
\text{CH}_3 \text{CH}_3 \text{CH}_3 \text{CH}_3 \text{CH}_3
\]

\[
\text{CH}_2\text{C(C(CH}_2\text{)}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_3
\]

\[
\text{HO-}P-OH
\]

**Intravenous Administration**

Vitamin K\textsubscript{1} emulsion and the disodium salt of 2-methyl-3-phytyl-1,4-naphthohydroquinone-1,4-diphosphate (dihydroyvitamin K\textsubscript{1} diphosphate) were compared at dosage levels of 1, 2, 5 and 10 mg./Kg. of vitamin K\textsubscript{1}, with regard to efficiency in reversing Dicumarol-induced hypoprothrombinemia. These two

\*Various specimens of, e.g., dihydroyvitamin K\textsubscript{1} diphosphate disodium did not necessarily give the same pH in aqueous solution. This indicates that the sodium content, and therefore the molecular weight also, may vary between samples. To correct for this effect, the concentration of these salts was calculated from the ultraviolet data, using the known molecular extinction of the free acids.
compounds were similarly compared in two experiments at 5 mg./Kg. for
efficacy in counteracting hypoprothrombinemia due to Dipaxin. Groups of
three dogs each were employed at each dose level of both compounds and
two or three dogs were used as controls receiving no vitamin K treatment.

Results
When given by the intravenous route, the two compounds were found to
possess approximately the same activity at equimolar dose levels, irrespective
of the anticoagulant used to induce hypoprothrombinemia. Activity was
usually manifest, at dosage levels of 2 mg./Kg. and above, within one hour
after injection, and after five hours prothrombin times were within a few
seconds of pretreatment control values. Figure 1 demonstrates the rapid
decrease in prothrombin time in Dicumarol-dosed dogs following the intravenous
administration of 2 mg./Kg. of the two test compounds. Different results were
obtained when the same total amount of Dicumarol was given in two doses
of 4 mg./Kg. each rather than in single doses of first 6 mg./Kg. and then
2 mg./Kg. As before, the prothrombin times dropped rapidly during the five-
hour period following vitamin K treatment. Two days later, however, the pro-
thrombin time of dogs given the water-soluble compound was again elevated
and it rose still higher two days after this. The prothrombin time of dogs
treated with the emulsion remained low and did not show such a recurrence
of anticoagulant effect (fig. 2). At the lowest level employed, namely 1
mg./Kg., a longer time was required for depression of the prothrombin time,
and "break throughs" or rebounds in prothrombin times occurred with both
the emulsion and water-soluble forms of vitamin K₁. These rebounds appeared
earlier and were greater in magnitude in the dogs injected with water-soluble
material.
Fig. 2.—Comparison of vitamin K₁ emulsion and dihydrovitamin K₁ diphosphate disodium salt (DK₁ Diphos) intravenously in Dicumarol-induced hypoprothrombinemia. Compare with figure 1.

Fig. 3.—Relative rates of action of vitamin K₁ emulsion and dihydrovitamin K₁ diphosphate disodium salt (DK₁ Diphos) intravenously in Dipaxin-induced hypoprothrombinemia.

The depression in prothrombin time at the one-hour interval after treatment was usually greater in dogs injected with the water-soluble K₁ compound than in those given K₁ emulsion. The effect of the emulsion became equivalent to that of the soluble compound, however, by the 2½ or 5-hour interval. Figure 3 shows this effect in Dipaxin-dosed dogs.

A comparison by the intravenous route of three water-soluble compounds at dosage levels of 2, 5 and 10 mg./Kg. showed the diphosphate and monophosphate derivatives of 2-methyl-3-phytyl-1,4-naphthohydroquinone to pos-
scess approximately equivalent activity and the 1-propionate-4-phosphate derivative to be less than one-half as active as these.

**Oral Administration**

Comparisons of the efficacies of vitamin K₁ emulsion and the disodium salt of dihydrovitamin K₁ diphosphate were conducted at levels of 5, 10 and 20 mg./Kg.

**Results**

The two preparations showed approximately equivalent activity. At the two higher dose levels, virtually complete counteraction of anticoagulant action occurred within a five-hour period following treatment. A rebound was observed 48 hours after treatment at the 10 mg./Kg. dosage level of water-soluble material (fig. 4). At the 5 mg./Kg. level, similar recurrences of anticoagulant effect occasionally occurred with both compounds.

The rate of action of the water-soluble diphosphate derivative, administered orally and intravenously, is shown in figure 5. It can be seen that intravenous treatment resulted in a precipitous decrease in prothrombin time in one hour, whereas a five-hour period was required for a similar decrease following oral administration.

**Intramuscular Administration**

The comparative efficacies of vitamin K₁ emulsion and dihydrovitamin K₁ diphosphate disodium salt were determined at dose levels of 5 and 10 mg./Kg. of vitamin K₁. Dicumarol was given orally at 6 mg./Kg. initially and at 4 mg./Kg. the following day. The K compounds were administered two days after the last dose of Dicumarol.

**Fig. 4.**—Comparison of vitamin K₁ emulsion and dihydrovitamin K₁ diphosphate disodium salt (DK₁ Diphos) orally in Dicumarol-induced hypoprothrombinemia.
Fig. 5.—Comparison of dihydrovitamin K₁ diphosphate disodium salt (DK₁ Diphos) intravenously and orally in Dicumarol-induced hypoprothrombinemia.

Results

Figure 6 shows the effect of treatment at the 10 mg./Kg. dose level. A moderate decrease in prothrombin time occurred within five hours in the dogs given the water-soluble preparation, whereas only a slight decrease was noted in the vitamin K₁ emulsion-treated animals at this time. Within 24 hours, the prothrombin time of dogs given water-soluble K₁ had attained pretreatment levels and no further change occurred. In contrast, the prothrombin time of animals which received vitamin K₁ emulsion dropped relatively little in the first 24 hours and required a period of 4 days for normalization. Results at the 5 mg./Kg. level were quite similar, but the initial five-hour decrease was relatively slight with both compounds. The prothrombin time decreased to within a few seconds of normal within 24 hours after water-soluble K₁, but, as before, a 4-day period elapsed before the emulsion-treated dogs attained pretreatment prothrombin times.

The relative inactivity of vitamin K₁ emulsion upon intramuscular administration suggested that the emulsion may have broken down. In order to examine this possibility, a group of normal rabbits was injected intramuscularly with the emulsion and a few were sacrificed on each of three consecutive days. Yellow, oily material was found at the injection site in every animal. Microscopic examination of this revealed numerous lipid particles, thus providing evidence that the emulsion had separated into its phases.

Refractoriness to Anticoagulant Action

A study was made of the relative propensities of vitamin K₁ emulsion and the water-soluble diphosphate compound to induce a refractory state when administered to dogs rendered hypoprothrombinemic by Dicumarol and Dipaxin. Dicumarol was administered at 6 mg./Kg. initially and at 2 mg./Kg.
the following day. Forty-eight hours after the second dose, the K compounds were given intravenously and 72 hours after this, Dicumarol was given as before at 6 and 2 mg./Kg.

Results

The prothrombin curves during the first Dicumarol-dosing period, both before and after administration of the two K compounds, were comparable. During the second Dicumarol-dosing period, however, the prothrombin time of the dogs previously treated with vitamin K₁ emulsion increased less rapidly and failed to attain values as high as those recorded for the first period. In contrast, no evidence of even mild refractoriness to anticoagulant was noted in the dogs treated with the water-soluble compound. In a similar experiment, Dipaxin was given on two consecutive days at 0.2 mg./Kg. The vitamin K preparations were administered three days later and three days after this a second course of two daily doses of Dipaxin at 0.2 mg./Kg. was given. Control dogs, receiving no vitamin K₁, were given Dipaxin only for the first period. The prothrombin curves of dogs treated with either K₁ emulsion or water-soluble material were virtually identical during the first Dipaxin-dosing period but were strikingly divergent in the second period (fig. 7). Thus the dogs given water-soluble material were at least as sensitive as before, whereas the emulsion-treated dogs were less responsive to the second course of Dipaxin administration.

Prophylactic Administration of Vitamin K Compounds

The greater incidence of rebounds and the absence of refractoriness in dogs treated with the water-soluble diphosphate derivative suggested that this material possesses a shorter duration of action than vitamin K₁ emulsion. To
obtain information on this point, dogs were given a combination of Dicumarol orally at 2 mg./Kg. and either K₁ emulsion or a water-soluble K₁ derivative at 1 mg./Kg. intravenously on each of four consecutive days. Prothrombin values were followed for a total period of 11 days.

Results

Vitamin K₁ emulsion administration prevented the prothrombin time from increasing more than a few seconds. The prothrombin curves of dogs given dihydrovitamin K₁-1-propionate-4-phosphate or no vitamin K were comparable, whereas the water-soluble diphosphate curve was approximately intermediate between that of the two extremes (fig. 8). The greater efficacy of vitamin K₁ over the water-soluble derivatives in preventing the onset of hypoprothrombinemia would appear to be ascribable principally to a longer persistence in the body.

Discussion

Hypoprothrombinemia, induced by either Dicumarol or Dipaxin, has previously been shown to be more readily reversed by emulsions of vitamin K₁ or K₂ oxide than by Menadione or its water-soluble derivatives. The present study in dogs reveals that certain water-soluble derivatives of vitamin K₁, namely the 4-monophosphate and the 1,4-diphosphate of 2-methyl-3-phytyl-1,4-naphthohydroquinone, are about as potent as vitamin K₁ emulsion. Comparisons of the diphosphate and emulsion, at equimolar dosage levels, and over short periods, showed these to possess equivalent potency by either the intravenous or oral route. More of these vitamin K₁ compounds is required orally (5-10 mg./Kg.) than intravenously (1-2 mg./Kg.) for comparable antidotal efficacy in the dog. A precise therapeutic comparison between species
is not feasible, but it appears that man requires less vitamin K₁, on a body weight basis, than does the dog. The superiority of the diphosphate over the emulsion in intramuscular therapy was clearly indicated. The relative inefficacy of vitamin K₁ emulsion by intramuscular injection in both dogs and humans has been reported previously by Shoskes.

At least a partial explanation for this is provided by the current finding that vitamin K₁ emulsion is not stable in muscle tissue. Water-soluble vitamin K₁ should be of value for intramuscular use in infants and the aged in whom intravenous and oral therapy is frequently infeasible. Collateral evidence in animals suggests that water-soluble vitamin K₁ is not likely to produce kernicterus in premature infants such as has been reported following intramuscular injection of water-soluble Menadione derivatives.

In patients receiving prothrombin-depressing anticoagulant therapy for thrombo-embolic disease, there are two major indications for vitamin K treatment, namely inordinate hypoprothrombinemia, with or without frank hemorrhage, and the need for surgery. After restoration of the prothrombin to a safe level, resumption of anticoagulant treatment is usually indicated. Careful control of vitamin K antidotal action and avoidance of a refractory state are therefore desirable. Refractoriness to anticoagulant can be circumvented by the use of relatively small doses of vitamin K₁ emulsion intravenously or preferably orally, but in an emergency such restrained therapy may be inadequate. Water-soluble vitamin K₁ may permit effective control of hypoprothrombinemia with little or no subsequent refractoriness because of its shorter duration of action. In the treatment of prothrombinopenia, due to obstruction of bile or to biliary fistula, it should be unnecessary to administer bile salts along with oral water-soluble vitamin K₁ derivatives such as is recommended in the case of oral therapy with vitamin K₁ itself.
The one-stage method of Quick for determining prothrombin time, as used in this investigation, reflects not only the plasma content of prothrombin but also of factors, such as proconvertin (factor VII, co-thromboplastin, stable factor), which accelerate the conversion of prothrombin to thrombin. It is recognized that the hypoprothrombinemia due to coumarin derivatives is accompanied by hypoproconvertinemia and that vitamin K, corrects both conditions. Although proconvertin was not measured in the present investigation, it can be deduced from the over-all effect that, if hypoproconvertinemia occurred in the anticoagulant-dosed dogs, it was counteracted promptly, along with hypoprothrombinemia, by both vitamin K, emulsion and water-soluble vitamin K,.

In vitro studies with mitochondrial suspensions have revealed that vitamin K, participates in the process of oxidative phosphorylation. A large percentage of cellular vitamin K is reported to be present in the mitochondrial fraction. It is anticipated that the availability of water-soluble vitamin K, derivatives may facilitate enzyme studies relating to the physiologic role of this vitamin and, in particular, to the role of the phytol group. The phytol group is not essential for activity in correcting vitamin K lack, but it appears to play an important role in conferring to K compounds a high order of potency against drug-induced hypoprothrombinemia. Thus the phytol-containing vitamin K, oxide and water-soluble vitamin K, derivatives exert much greater antidotal action against coumarin and indandione compounds than do Menadione and its water-soluble derivatives which lack the phytol group. Reported studies suggest that Menadione is a provitamin and is converted into Vitamin K, in the body by the addition of the phytol group. One might speculate, on this basis, that the physiologic conversion of Menadione to vitamin K, proceeds with relative ease in simple deficiency states but is inhibited to a variable degree in the presence of hypoprothrombinemia-producing anticoagulants.

Terms such as “vitamin K” or “water-soluble vitamin K” have been used very loosely in some publications. It is clearly evident that a number of compounds possess vitamin K type activity and also that a given compound may vary considerably in potency, depending upon test conditions. It would seem highly desirable, therefore, that the term “vitamin K” be employed only in a general sense when referring to the type of activity or in instances (e.g., concentrates or tissue homogenates) of uncertain identity. Use of the chemical name, product name, or even a readily recognizable abbreviation is recommended. At the very least, it should be indicated whether the compound under consideration is a vitamin K, or Menadione derivative or analogue.

SUMMARY

The relative efficacies of vitamin K, emulsion and several water-soluble derivatives of vitamin K, were determined in dogs rendered hypoprothrombinemic by Dicumarol and Dipaxin.

In short term experiments equimolar intravenous doses of the disodium salt of 2-methyl-3-phytyl-1,4-naphthohydroquinone-1,4-diphosphate and of vitamin
K₁ emulsion exhibited like activity in reversing Dicumarol- and Dipaxin-induced hypoprothrombinemia. By this route, the 4-monophosphate derivative showed comparable activity to the 1,4-diphosphate, but the 1-propionate-4-phosphate derivative was less potent.

At equimolar dosage levels, the water-soluble diphosphate derivative was found to possess oral activity equivalent to that of vitamin K₁ emulsion. It was appreciably more effective than the emulsion, however, by the intramuscular route.

With both K₁ emulsion and the water-soluble diphosphate derivative, the most rapid effect followed intravenous administration. Oral treatment was less rapid and intramuscular the slowest.

Data obtained in prophylactic and therapeutic studies indicate that water-soluble vitamin K₁ has a shorter duration of action than vitamin K₁ emulsion. Because of this property, it should be possible to control treatment more precisely with water-soluble vitamin K₁ and also to effect a rapid reversal of excessive hypoprothrombinemia with less liability of including refractoriness to reinstitution of anticoagulant therapy.

SUMMARIO IN INTERLINGUA

Le relative efficacia contra-active de emulsion de vitamina K₁ e de plure hydrosolubile derivatos de vitamina K₁ esseva determinate in canes rendite hypoprothrombinemic per medio de Dicumarol e Dipaxin.

In experimentos a breve vista, equimolar doses intravenose del sal dinatrium 2-methyl-3-phytyl-1,4-naphthohydroquinona-1,4-diphosphatic e del emulsion de vitamina K₁ exhibiva le mesme efficacia in reverter hypoprothrombinemia induite per Dicumarol e Dipaxin. Administrate per iste via, le derivato 4-monophosphatic monstrava un activitate comparable a illo del 1,4-diphosphato, sed le 1-propionato-4-phosphato esseva minus potente.

A equimolar dosages, il esseva trovate que le hydrosolubile derivato diphosphatic possedeva un activitate oral equivalente a illo de emulsion de vitamina K₁. Tamen, in administrationes per via intramuscular, le diphosphato esseva appreciablemente plus efficace que le emulsion.

Tanto in le caso del emulsion de vitamina K₁ como etiam in le caso del hydrosolubile derivato diphosphatic, le plus rapide effecto resultava del administration intravenose. Le tractamento oral esseva minus rapide. Le tractamento intramuscular esseva le plus lente.

Observationes in studios prophylactic e therapeutic indica que le forma hydrosolubile de vitamina K₁ ha un plus breve action que le emulsion de vitamina K₁. Per consequente, le uso del forma hydrosolubile deberea permetter un plus precisemente regulate tractamento e etiam le effectuation plus rapide del reversion de grados excessive de hypoprothrombinemia sin grande risco de inducer refractorietate al reinstitution del therapia anticoagulante.

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Efficacy of Water-Soluble Derivatives of Vitamin K₁ in Counteracting Drug-Induced Hypoprothrombinemia

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