THE IN VITRO EFFECT OF ANALOGS OF VITAMIN K ON
MYCOBACTERIUM TUBERCULOSIS VAR. HOMINIS,
STRAIN H37Rv

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Phthiocol, the pigment isolated from Mycobacterium tuberculosis var. hominis, strain H37, by Anderson and Newman (1933a), has a structural formula identical with that of vitamin K, except for the substitution of a hydroxyl group for the phytyl radical on its third carbon. The synthesis of this antihemorrhagic vitamin by Mycobacterium tuberculosis var. hominis was noted in animal experiments by Alquist, Pentler, and Necchi (1938). In the following year Alquist and Klose (1939a) tested the activity of pure synthetic phthiocol and showed that its properties were generally similar to those of vitamin K. The same authors, evaluating other synthetic and natural antihemorrhagic compounds (1939b), stated that the activity nucleus of each was 2-methyl-1,4-naphthoquinone, and that they differed from phthiocol by the absence of a hydroxyl group on the third carbon.

Because compounds with this activity nucleus are chemically related to phthiocol, an investigation of the in vitro effect of analogs of vitamin K on Mycobacterium tuberculosis var. hominis, strain H37Rv, was undertaken.

MATERIALS AND METHODS

Liquid medium. The medium chosen for the in vitro tests was the simple chemically defined one of Youmans (1944). It has the following composition: asparagine, 0.5 per cent; KH₂PO₄, 0.5 per cent; Mg₃(C₆H₅O₇)₂, 0.25 per cent; K₂SO₄, 0.05 per cent; and glycerol, 2 per cent. Sufficient water was added so that the final concentration of ingredients, after the addition of the analog, would be as given above. The pH was adjusted to 7.1 and aliquots of 4.9 ml were added to screw-capped, plastic-lined, clear-glass, round bottles measuring 25 mm by 100 mm. Sterilization was effected by autoclaving at 10 pounds of pressure for 20 minutes.

Analogs of vitamin K. Stock solutions of each of the four analogs of vitamin K tested consisted of sterile aqueous packaged ampoules. The dilution of each, effected under aseptic precautions, was such that the addition of 0.1 ml of the analog used to 4.9 ml of the sterilized medium resulted in the required concentration. Tetrasodium 2-methyl-1,4-naphthoquinone diphosphoric acid ester or "synkayvite" (Hoffmann-LaRoche) comes in ampoules of 10 mg per ml; 2-methyl-1,4-naphthoquinone sodium bisulfite or "hykinone" (Abbott) is packaged in 10-ml ampoules of 72 mg; 4-amino-2-methyl-1-naphthol hydrochloride or "synkamin" (Parke, Davis) is supplied as ampoules of 1 mg per ml; and "kappadione" (Lilly), which has a structural formula identical to that of "synkayvite," also comes as a 10-mg-per-ml ampoule.
Test organism and inoculum. The H37Rv strain of Mycobacterium tuberculosis var. hominis was supplied by the National Tuberculosis Association, Trudeau, New York. Before the experimental work was begun, the organism was acclimated to the medium by subcultures at biweekly intervals. The inoculum was prepared by emulsifying a 2 weeks' growth of the organism with glass beads and then centrifuging it to remove the gross particles. Direct microscopic counts were made of 0.01 ml of the bacterial suspension, and it was then diluted to give a count of between 10,000 to 20,000 bacteria per 0.01 ml. This constituted the inoculum.

Evaluation of results. Examination for inhibition of growth was made after 2 weeks' incubation at 37 C. Growth was measured in terms of "plus," ranging from one to a maximum of four pluses for the positive controls consisting of the inoculated unaltered basic medium, and minus was used for no growth.

The "absolute concentration" of the analogs tested was the lowest concentration in μg per ml giving complete inhibition of growth of the organism. The "critical concentration" was the lowest concentration in μg per ml giving 50 per cent inhibition or a two-plus reading when compared to the controls.

RESULTS

Table 1 shows the effective concentrations of the four analogs of vitamin K tested. "Synkayvite" and "kappadione" failed to completely inhibit the multiplication of the organism in the highest concentration used, which accounts for the absence of absolute concentration figures (table 1). As is obvious from table 1, there is a marked difference in the effect of both "synkayvite" and "kappadione" in comparison with that of "synkamin" and "hykinone." The latter are much more effective than the former as tuberculostatic agents, in vitro. "Synkamin" was the most effective in that its critical concentration was 1 μg per ml and its absolute concentration 2 μg per ml. "Hykinone" showed a critical concentration of 2 μg per ml with 10 μg per ml as the absolute concentration. Both "synkayvite" and "kappadione" had a critical concentration of 100 μg per ml.

DISCUSSION

An analysis of the structural formulae of the four analogs of vitamin K tested, with that of 2-methyl-1,4-naphthoquinone and phthiocol, indicates the
possible basic principles in operation that probably explain the observed phenomena. One notes the similarity of the basic pattern in figure 1. Phthiocol differs from 2-methyl-1,4-napthoquinone in the presence of a hydroxyl group on the third carbon. The 2-methyl-1,4-napthoquinone is the activity nucleus about which the analogs of vitamin K are constructed. It would appear as if the analogs of vitamin K were functioning as inhibitory competitive analogs of the metabolite, phthiocol. The validity of this assumption is further emphasized by the observations of Woolley and McCarter (1940) that extracts of Mycobacterium phlei, phthiocol, and 2-methyl naphthoquinone were all effective in promoting growth of Mycobacterium paratuberculosis (Johne's bacillus). Woolley (1945), working with Saccharomyces cerevisiae, created an inhibitory structural analog of vitamin K, 2,3-dichloronaphthoquinone, and stated that "over a limited range of concentration the antagonism between them was competitive."

"Synkamin" was the most effective of the analogs tested. This could be explained by its possession of an amino group on the fourth carbon. Freedlander and French (1947), working with benzothiazole derivatives, tested their effect in experimental tuberculosis. They stated that an invariant characteristic of the
compounds studied was the possession of a free amino group in the 6 position. Eiseman (1948), in attempting to increase the tuberculostatic effect of sulfonamide derivatives by attaching the hydrophobic end of the molecule to a surface-active molecule, stated that a free amino group was required for optimum action. The general antibacterial properties of "synkamin" were discussed by Schwartzman (1948). He too stated that the presence of an amino substituent group played an important role in the antibacterial activity described. It would appear that the possession of a free amino group increases the tuberculostatic property of a compound.

**SUMMARY**

The *in vitro* tuberculostatic effect of four analogs of vitamin K, i.e., 4-amino-2-methyl-1-naphthol hydrochloride ("synkamin"), 2-methyl-1,4-naphthoquinone sodium bisulfite ("hykinone"), and tetrasodium 2-methyl-1,4-naphthoquinone diphosphoric acid ester ("synkayvite" and "kappadione"), were studied. The critical concentration for "synkamin" was 1 μg per ml, for "hykinone" 2 μg per ml, and for "synkayvite" and "kappadione" 100 μg per ml. A possible explanation for their effect, and for the differences in their effect, on *Mycobacterium tuberculosis* var. *hominis* H37Rv is presented.

*REFERENCES*


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