15693

Antagonism of the Hemorrhagic Syndrome Induced by Derivatives of 3-Hydroxy-1,4-Naphthoquinone.*

CARL C. SMITH. (Introduced by L. H. Schmidt.)

From the Institute of Medical Research, The Christ Hospital, Cincinnati, Ohio.

It has been reported recently[1] that certain 2-substituted-3-hydroxy-1,4-naphthoquinones, when administered to the white rat, induce a hemorrhagic syndrome which is associated with a hypoprothrombinemia. Preliminary studies showed that the hemorrhagic activities of at least one of these naphthoquinones, 2-(3-cyclohexylpropyl)-3-hydroxy - 1,4-naphthoquinone (SN 5090†), could be antagonized completely by simultaneous administration of vitamin K_1. Similar administration of 2-methyl-1,4-naphthoquinone had less marked effect. Subsequent to this work experiments were carried out on 2 other 2-substituted-3-hydroxy derivatives. When it was found that these compounds, like the naphthoquinones described previously,[1] also induced a hemorrhagic syndrome and hypoprothrombinemia it was decided to expand the previous observations on the antagonistic action of vitamin K_1 and 2-methyl-1,4-naphthoquinone. The present report deals primarily with this phase of the study.

Experimental. The compounds studied included 2-cyclohexyl-3-hydroxy-1,4-naphthoquinone (SN 5094), 2-(2-methyloctyl)-3-hydroxy-1,4-naphthoquinone (SN 5949), and 2-[3 - (*p*-phenoxyphenyl)propyl]-3-hydroxy-1,4-naphthoquinone (SN 13,936‡). All 3 of these naphthoquinones induced a hemorrhagic syndrome characterized by gross extravasations of blood into the subcutaneous tissues of the limbs, head, neck and scrotum, and diffuse bleeding of the gastrointestinal mucosa; associated with this syndrome were greatly prolonged clotting and prothrombin times. SN 5094 was the least toxic of the compounds; daily doses of 400 mg per kg uniformly produced fatal lesions but doses of 200 mg had little effect. SN 5949 was somewhat more toxic; it usually produced fatal lesions at 200 mg per kg but had little effect at lower doses. SN 13,936 was the most toxic of the compounds; it uniformly was fatal at 200 mg per kg doses and produced severe hemorrhages at doses of 100 mg.

The technics employed in studying the antagonistic effects of vitamin K_1 and 2-methyl-1,4-naphthoquinone were similar to those used in the previous study.[1] Male rats, 4 to 5 weeks old, Sprague-Dawley strain, were used throughout and were fed a diet of Purina Dog Chow checkers *ad libitum.*

The drugs and vitamin adjuncts were suspended or dissolved in olive oil and were administered once daily via stomach tube. Treatments were continued for 8 to 12 days where the rats survived that long. Whenever it was possible to obtain blood samples, prothrombin times were measured. Determinations were carried out on whole blood using the method of Kato[2] with Russell viper venom as the source of thromboplastin. The results of the pertinent experiments have been summarized in Table I.

In the experiments with SN 5094, daily doses of vitamin K_1 of 10 mg per kg completely prevented the development of the hemorrhagic syndrome. Similar doses of 2-methyl-1,4-naphthoquinone were somewhat less effective. Hemorrhagic lesions appeared on the 4th day of the experiment but appeared to regress with continued treatment. At the end of the experiment normal pro-

* The work described in this paper was done under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the Institute of Medical Research, The Christ Hospital, Cincinnati, Ohio.

1 Smith, Carl C., Fradkin, R., and Lackey, M. D., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 398.

† The numbers cited here are those assigned to the compounds by the Survey of Antimalarial Drugs, Baltimore, Md.

‡ The drugs were prepared by Dr. Louis F. Fieser, Harvard University, and the Abbott Laboratories, to whom we are greatly indebted.

2 Kato, K., *Am. J. Clin. Path.*, 1940, **10**, 147.

TABLE 1.

The Effectiveness of 2-Methyl-1,4-Naphthoquinone and Vitamin K_1 on the Hemorrhagic Activities of SN 5094, SN 5949, and SN 13,956.

Rat No.	Daily treatment, mg/kg body wt	Days treatment	Effects of treatment: Prothrombin time, sec	Effects of treatment
1	400 mg SN 5094	4	—	Died 4th day; typical hemorrhagic lesions.
2		4	—	" " " " " "
3		5	—	" 5th " " " "
4		6	—	" 6th " " " "
5		8	—	" 8th " " " "
6	400 mg SN 5094 and	6	—	" 6th " " " "
7	10 mg 2-methyl-1,4-	11	30	Hemorrhagic lesions on limbs.
8	naphthoquinone	11	23	" " " "
9		11	17	Regressing hemorrhagic lesion in scrotum.
10	400 mg SN 5094 +	11	20	No hemorrhagic lesions.
11	10 mg vit. K_1	11	18	" " "
12		11	17	" " "
13		11	14	" " "
14	200 mg SN 5949	3	—	Died 3d day; no hemorrhagic lesions.
15		5	25*	Hemorrhagic lesions on limbs and head.
16		8	190	" " " " " scrotum.
17		8	50	No hemorrhagic lesions.
18	200 mg SN 5949 and	4	—	Died 4th day; no hemorrhagic lesions.
19	10 mg 2-methyl-1,4-	8	35	Regressing hemorrhagic lesions on limbs.
20	naphthoquinone	8	30	No hemorrhagic lesions.
21		8	25	" " "
22	200 mg SN 5949 and	8	15	" " "
23	10 mg vit. K_1	8	12	" " "
24		8	11	" " "
25		8	10	" " "
26	200 mg SN 13,936	3	—	Died 3rd day; gastrointestinal hemorrhages.
27		4	—	" 4th " typical hemorrhagic lesions.
28		5	>600	" 5th " " " "
29		6	—	" 6th " " " "
30		8	>600	" 8th " " " "
31	200 mg SN 13,936 and	3	—	" 3rd " gastrointestinal hemorrhages.
32	10 mg 2-methyl-1,4-	6	—	" 6th " typical hemorrhagic lesions.
33	naphthoquinone	9	—	" 9th " " " "
34		10	—	" 10th " " " "
35	200 mg SN 13,936 and	11	30	No hemorrhagic lesions.
36	10 mg vit. K_1	11	40	" " "
37		11	60	" " "
38		11	140	" " "
39	200 mg SN 13,936 and	12	12	" " "
40	100 mg vit. K_1	12	14	" " "
41		12	15	" " "
42		12	18	" " "

* Prothrombin time obtained on 7th day. Animal too ill for treatment after 5th day.

thrombin times were obtained.

The findings with SN 5949 were similar to those just described. Vitamin K_1 in 10 mg per kg doses prevented the development of hemorrhagic lesions and hypoprothrombinemia whereas only a partial effect was obtained with this dosage of 2-methyl-1,4-naphthoquinone.

In the experiments with SN 13,936, 10 mg doses of 2-methyl-1,4-naphthoquinone were

without apparent effect on the syndrome. Even vitamin K_1 at 10 mg per kg doses did not completely block the action of SN 13,936 for, although hemorrhagic lesions were absent, a significant hypoprothrombinemia was present. At 100 mg per kg doses, however, vitamin K_1 blocked development of both the hemorrhagic lesions and the hypoprothrombinemia.

Discussion. The data presented above demonstrate that, as in the case of SN 5090,† vitamin K_1 and to a lesser extent 2-methyl-1,4-naphthoquinone antagonize the hemorrhagic syndrome and hypoprothrombinemia induced by SN 5094, SN 5949 and SN 13,936. This finding would seem to strengthen the suggestion that the 2-substituted-3-hydroxy-naphthoquinones exert their hemorrhagic activity by competing with the K vitamins in whatever processes these substances serve in prothrombin formation.

This suggestion appears to offer the only tenable explanation for the hemorrhagic activity of the above naphthoquinones. The compounds do not produce severe liver injury, nor are they especially active antibacterial agents which might interfere with the synthesis of K vitamins by intestinal bacteria. Furthermore, extensive *in vitro* experiments indicate that they do not inactivate prothrombin as do the indandiones.[3] It appears, therefore, as if the relationship of the 2-substituted-3-hydroxy-1,4-naphthoquinones to vitamin K affords another example of antagonism of metabolites by structurally related compounds.

Summary. The development of the hypoprothrombinemia and hemorrhagic lesions, which occurred in the albino rat as the result of ingestion of various 2-substituted-3-hydroxy-1,4-naphthoquinones, could be prevented either partially or completely by simultaneous administration of 2-methyl-1,4-naphthoquinone or vitamin K_1. The amounts of these K vitamins required to block development of the above syndrome differed with the various hydroxy-naphthoquinone derivatives. In each instance, however, vitamin K_1 proved more effective than 2-methyl-1,4-naphthoquinone.

Observations made in this report confirm our earlier suggestion that the hemorrhagic syndrome, which results from administration of 2 - substituted - 3 - hydroxy-1,4-naphthoquinones, is due to competition between these substances and the K vitamins in processes involved in prothrombin formation.

[3] Kabat, H., Stohlman, E. F., and Smith, M. I., *J. Pharm. and Exp. Therap.*, 1944, **80**, 160.

15694

Action of 3,3′ Methylenebis (4-Hydroxycoumarin) (Dicumarol) on Thromboplastic Activity of Rabbit Brain.*

M. STEFANINI† AND M. C. BLANCHAER.‡ (Introduced by Armand J. Quick.)

From the Department of Biochemistry, School of Medicine, Marquette University, Milwaukee, Wisconsin.

Several observations suggest that other factors besides the interference with the formation of prothrombin[1] must be considered in the pathogenesis of the bleeding tendency produced by dicumarol. Widespread dilatation of capillaries, arterioles and venules has been found, indicating that the hemorrhagic condition is due, at least in part,

* This research was supported by a grant from the United States Public Health Service.

† Assistant, Medical Clinic of the University of Rome; at present, Research Fellow, Institute of International Education.

‡ Research Fellow, Department of Biochemistry, School of Medicine, Marquette University, Milwaukee, Wisc.

[1] Quick, A. J., *The Hemorrhagic Diseases*, pp. 284-285, C. C. Thomas, Springfield, Ill., 1942.