ANTICOAGULANT ACTIVITY OF METHYLATED COUMARIN DERIVATIVES

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Summary

Tremendous research on coumarin derivatives and anticoagulant drug are main inspiration of this work. Methylated coumarin derivative substituted by alkylaminohaydroxypropoxy side chain gives antihypertensive as well as anticoagulant activities. Therefore, in the present project, we attempted to synthesize a single drug molecule with antihypertensive activity of propranolol and anticoagulant activity of warfarin (coumarin).

Key words: Anticoagulant, methylated coumarin, antihypertensive

Introduction

It may be a first attempt to work on coumarin derivative as antihypertensive agent. Blood is a specialized bodily fluid that delivers necessary substances to the body's cells – such as nutrients and oxygen – and transports waste products away from those same cells. Blood is composed of blood cells suspended in a liquid called blood plasma. Plasma, which comprises 55% of blood fluid, is mostly water (90% by volume) and contains dissolved proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being main medium for excretory product transportation), platelets and blood cells themselves. The blood cells present in blood are mainly red blood cells (also called RBCs or erythrocytes) and white blood cells, including leukocytes and platelets.

The most abundant cells in human blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates transportation of oxygen by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is almost entirely transported extracellular dissolved in plasma as bicarbonate ion [1].

Blood diseases affect the production of blood and its components, such as blood cells, hemoglobin, blood proteins, mechanism of coagulation, etc. Thrombosis is formation of a blood clot (thrombus) inside a blood vessel, obstructing flow of blood through circulatory system. When a blood vessel is injured, body uses platelets and fibrin to form a blood clot, because first step in repairing it (hemostasis) is to prevent loss of blood. If that mechanism causes too much clotting, and clot breaks free, an embolus is formed**.** This plug obstructs normal flow of blood and can result in a heart attack or stroke [2].

Warfarin is coumarin containing anticoagulant. The story of the coumarin anticoagulants generally is traced back to the early 1920s, when the "sweet clover disease" showed up almost simultaneously in North Dakota and in Alberta, Canada. This new malady of cattle involving fatal bleeding was traced to stacks of sweet clover hay [3]. An anticoagulant is a substance that prevents coagulation; that is, it stops blood from clotting. Anticoagulants were introduced into medical practice more than three decades ago. Extensive use of these drugs in the prevention and treatment of thromboembolic disease has made them one of the most widely used classes of pharmacological agents. Antiplatelet agents, anticoagulant agents and thrombolytic drugs use as antithrombic agent.

Coumarins are a group of important natural compounds, and have been found to have multi-biological activities such as anti-HIV, anti-tumor, anti-hypertensive, anti-arrhythmia, anti-osteoporosis, pain relief, preventing asthma and antisepsis [4]. Natural products like esculetin, fraxetin, daphnetin and other related coumarin derivatives are recognized as inhibitors not only of the lipoxygenase and cycloxygenase enzymic systems, but also of the neutrophil-dependent superoxide anion generation. Coumarin

derivatives also possess anti-inflammatory as well as antioxidant activities. Coumarin possesses immunomodulatory and direct antitumor activity [5]. It has been recommended for treatment of a number of clinical conditions, including high protein oedema and brucellosis. Coumarin and some of its derivatives have been tested for treatment of anxiolytic, microcirculation disorders and angiopathic ulcers, and also for treatment of high protein oedemas in animals [6].

Materials and Methods

Sterile disposable pricking needle, stop watch, dry glass capillary tube (narrow diameter 1 top 2 mm, minimum 10 cm long), cotton swab, spirit wetted, 70 % v/v ethyl alcohol.

Blood sample collection and blood analysis: Blood samples were collected in clean dry centrifuge tubes as end of three weeks of treatment after 12hrs fast from retro orbital plexuses under light ether anesthesia and were collected in EDTA tube to prevent clot formation at room temperature.

We have used Lee and white method for determination of clotting time.

Experimental

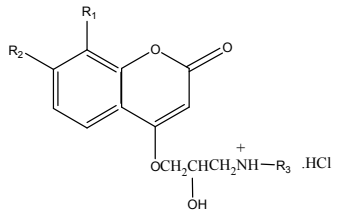
We have synthesized a series of methylated coumarin substituted derivatives and performed its pharmacological screening for anticoagulant activity. 4^{th} position is substituted with alkylaminohydroxypropoxy side chain as in propranolol.

Blood was collected from animal by retro orbital plexus method under light anesthetic conditions. Immediately stop watch was started. Dip one end of capillary into blood drop gently without pressure. After every 30 seconds, using stopwatch, break a small piece of capillary. Repeat breaking at regular time intervals, till fibrin thread appears at broken end of capillary tube. Do not pull away the cut pieces ling apart and bristly. Record time interval between pricking finger and first appearance of fibrin thread at the broken ends of capillary tube. That is clotting time of blood.

Statistical analysis: Results are presented as mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$. Statistical analysis was performed using Sigma stat statistical software.

Result and Discussion

We have synthesized a series of coumarin derivative. This may possess antihypertensive activity too.



Tab: 1 Synthesized compounds

Sr. No	Code	R_1	R_2	R_3	M.F.	M. P. (°C)	R_{f*} value	% Yield
1	BLT 11	$-CH_3$	H	$-CH(CH_3)_2$	$C_{16}H_{22}NO_4Cl$	245-47	0.31	56
2	BLT 12	$-CH_3$	H	$-C(CH_3)_3$	$C_{17}H_{24}NO_4Cl$	240-42	0.30	59

3	BLT 17	$-CH_3$	H	$-(C_2H_5)_2$	$C_{17}H_{24}NO_4$ Cl	270-72	0.31	50
4	BLT 18	$-CH_3$	H	$-(C_2H_40H)_2$	$C_{17}H_{24}NO_6$ Cl	258-60	0.40	38
5	BLT 20	$-CH_3$	H	$-(CH_3)_2$	$C_{15}H_{20}NO_4$ Cl	262-64	0.31	38
6	BLT 21	H	$-CH_3$	$-CH(CH_3)_2$	$C_{16}H_{22}NO_4$ Cl	251-53	0.31	50
7	BLT 22	H	$-CH_3$	$-C(CH_3)_3$	$C_{17}H_{24}NO_4$ Cl	244-46	0.24	48
8	BLT 27	H	$-CH_3$	$-(C_2H_5)_2$	$C_{17}H_{24}NO_4$ Cl	278-80	0.35	48
9	BLT 28	H	$-CH_3$	$-(C_2H_4OH)_2$	$C_{17}H_{24}NO_6$ Cl	258-60	0.41	38
10	BLT 30	H	$-CH_3$	$-(CH_3)_2$	$C_{15}H_{20}NO_4$ Cl	265-67	0.25	36

Warfarin treated (0.1mg/kg p.o.) rats were found to be shown significant increase in bleeding and clotting time as compare to normal healthy rat. Treatment with tested compounds (5ml/kg/day, p.o) also produced significant increase in bleeding and clotting time as compared to normal rats. We have not found it's mechanism but it is confirmed that change in chemical structure of coumarin side chain altered its anti-coagulant activity. Coumarin nucleus is responsible for anticoagulant activity. While side chain play important role in other activities like hypertension, arrhythmia etc. Our first aim is synthesized anticoagulant compound which posses antihypertensive activity. Our work may helpful to discover the new series of drug use in hypertension.

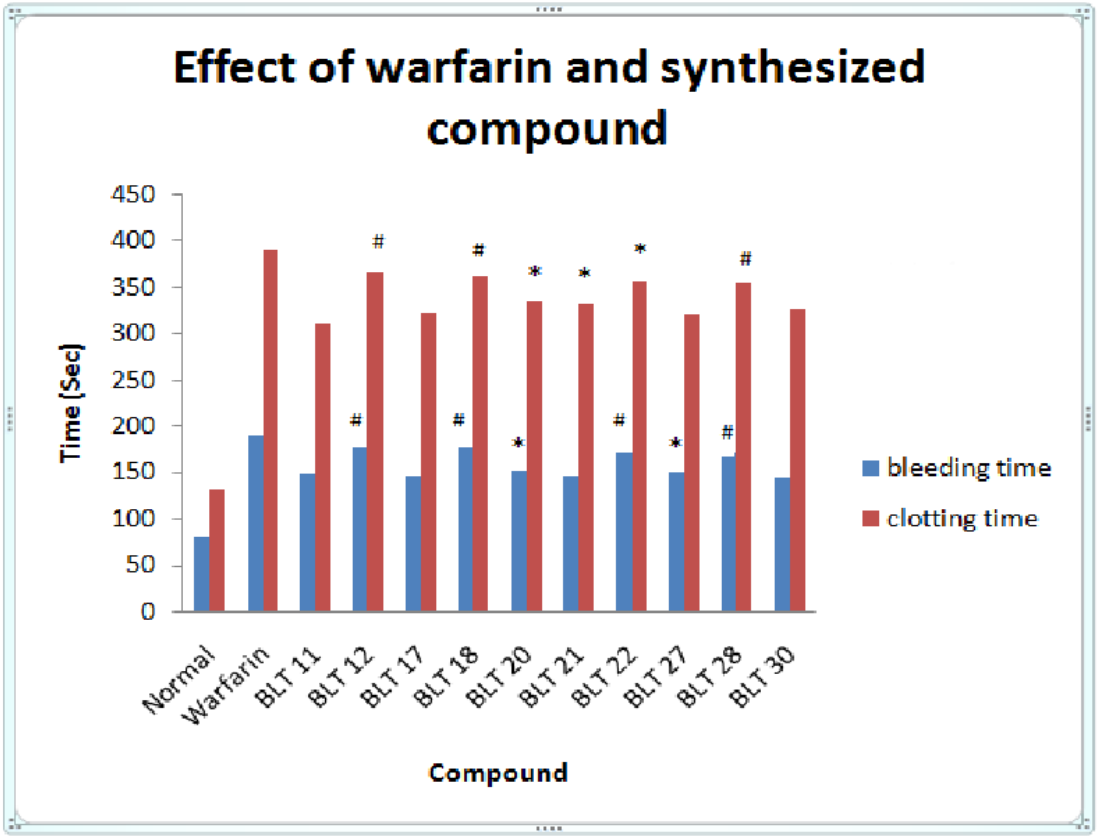
Tab: 2 Effect of warfarin and synthesized compounds on bleeding and clotting times on rats.

Blood parameters	Bleeding Time (sec)	Clotting Time (sec)
Normal	80+ 12	130+ 22
Warfarin	190+ 18 #	390+ 35 #
BLT 11	148 ± 25	310 ± 20
BLT 12	176 ± 28 #	365 ± 37 #
BLT 17	146 ± 37	322 ± 43
BLT 18	176 ± 43 #	362 ± 30 #
BLT 20	152 ± 24 *	335 ± 52 *
BLT 21	145 ± 35	331 ± 47 *
BLT 22	170 ± 23 #	356 ± 52 *
BLT 27	149 ± 35 *	320 ± 37
BLT 28	171 ± 12 #	358 ± 29 #
BLT 30	144 ± 39	325 ± 43

Values are expressed as Mean + S.E.M

*- significantly different from control ($p < 0.05$), # - significantly different from control ($p < 0.01$)

Fig: 1 Pharmacological Screening of anticoagulant activity



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