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SERUM LIPID-LOWERING EFFECT OF VINCA ALKALOIDS

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Abstract—The serum lipid-lowering effect of monomeric and dimeric forms of Vinca alkaloids was studied on normal mice and on those with hyperlipidemia induced by ascites tumours (Ehrlich, NK/Ly). The decrease of serum lipid level after a single, non-toxic dose of these agents proved to be quick and reversible and there was no correlation between the hypolididemic effect and antitumour activity of drugs.

Vinca alkaloids reduced first of all the neutral lipid (triglyceride) and very low density lipoprotein (VLDL) content of the serum. Decrease in VLDL level showed logarithmic correlation to the Vincristine dose applied. Simultaneously, higher doses increased the total lipid and triglyceride content of the liver.

Recently, Vinca alkaloids have been found to affect, in addition to their well known antitumour effect the lipid metabolism too [1–3]. In earlier studies we also observed that Vincristine evoked a rapid but reversible serum lipid decrease in hyperlipidemias induced by ascites tumours or Triton WR-1339 [4, 5]. There is some information about the effect of Vinca alkaloids on the cell membrane [6–8]. It has also been demonstrated that they interfere with different intracellular processes, inhibiting for example the VLDL secretion of hepatocytes [9, 10], presumably through the dissociation of the Golgi apparatus or the tubular system [11–13].

The present experiments were performed to elucidate the possible correlation between the chemical structure of Vinca alkaloids and their effect on the lipid metabolism as well as their tumour inhibitory and hypolipidemic effects. Therefore, monomeric and dimeric forms of Vinca alkaloids with and without antitumour activity were selected. Thorough analysis of the serum lipid-lowering effect of Vincristine was performed in tumour-bearing hyperlipidemic mice. The lipid content and composition of the liver of drug treated animals was determined simultaneously.

MATERIALS AND METHODS

Experiments were carried out with Swiss/H-Riop outbred male mice kept on a standard diet and water *ad libitum*. Body weight ranged from 30 to 35 g. The animals did not starve prior to the experiments.

The serum lipid-lowering effect of Vinca alkaloids was studied in normal mice and in those with hyperlipidemia induced by Ehrlich and NK/Ly ascites tumours. The two tumours did not reveal any noticeable difference concerning the development of hyperlipidemia or hypolipidemic effect on Vinca alkaloids. Hyperlipidemia was induced on 20–25 animals by intraperitoneal transplantation of 5×10^6 ascites tumour cells. The serum lipid level was measured daily and individually. Total lipid content of serum samples taken from the tail by heparinized capillary tubes was determined by the micromethod of Woodman and Price [14]. In earlier studies we observed distinct stages in the endogenous fat mobilization induced by ascites tumours [15]. Therefore, selection of animals for drug treatment on the 10–12th day after tumour transplantation was based on (a) nearly identical degree of hyperlipidemia, (b) obligatory rise in serum lipid level in comparison with the previous day's value. Single doses of Vinca alkaloids represented in Tables 1 and 2 were injected intraperitoneally in 10 ml per kg physiological saline solution. Acute toxicity values for each drug were determined during a 21-day-long observation period after the administration of single i.p. doses. All the alkaloids examined were provided by Chemical Works of Gedeon Richter Ltd., Budapest, Hungary.

The time dependence of the serum lipid-lowering effects of Vinca alkaloids was followed up by measuring the serum lipid and lipoprotein content 2, 4, 6, 8, 10, 12, 24 and 48 hr after i.p. treatment. Concentrations of the main serum lipid fractions (e.g. triglycerides, phospholipids, cholesterol, cholesterol esters and free fatty acids) were measured in pooled samples by quantitative thin-layer chromatography [16]. Serum lipoproteins were prestained with Sudan black B according to Ribeiro and McDonald [17], then separated to very low density (VLDL), low density (LDL) and high density (HDL) fractions by polyacrylamide gel electrophoresis containing 3.7, 5 and 10 per cent concentration gradient steps according to the method of Creinin and Narayan [18] slightly modified by us [15]. Electrophoretic patterns were directly registered by Photovolt Varicord 42B type densitometer with a filter of 610 nm. Concentrations of the lipoprotein fractions were expressed in mg lipoprotein–lipid per 100 ml serum.

Total lipid content of the liver after homogenization was extracted according to Folch *et al.* [19] then quantitated by weight. Protein content of liver homogenates was determined by the method of Hartree [20]. Triglyceride content of lipids in the liver was measured by quantitative thin-layer chromatography [16]. Total lipid and triglyceride levels in the liver were related to the protein content of the liver tissue.

RESULTS

Serial measurements of serum total lipids in normal and tumour bearing mice clearly demonstrated that all monomeric and dimeric forms of Vinca alkaloids

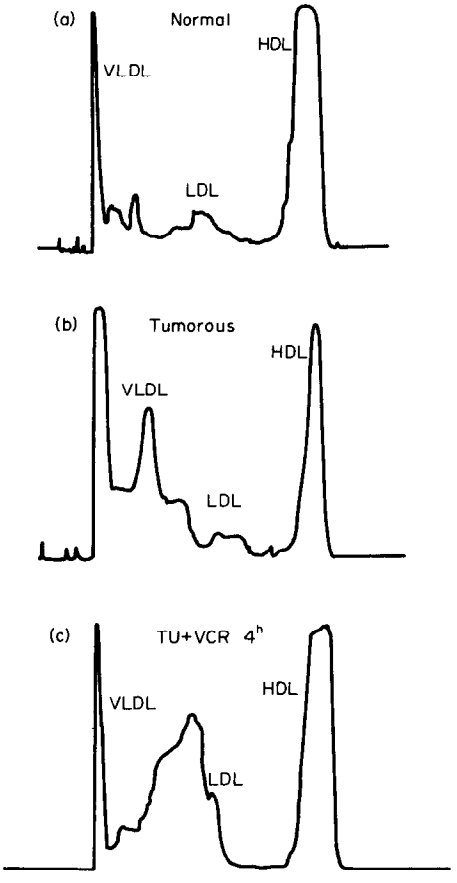

Fig. 1. Photodensitometry of polyacrylamide gel electrophoresis of serum lipoproteins in normal (a) and Ehrlich ascites tumour bearing mice untreated (b) and 4 hr after i.p. administration of 0.2 mg per kg Vincristine sulfate (c). Abbrevations: VLDL, very low density; LDL, low density; and HDL, high density lipoprotein fractions.

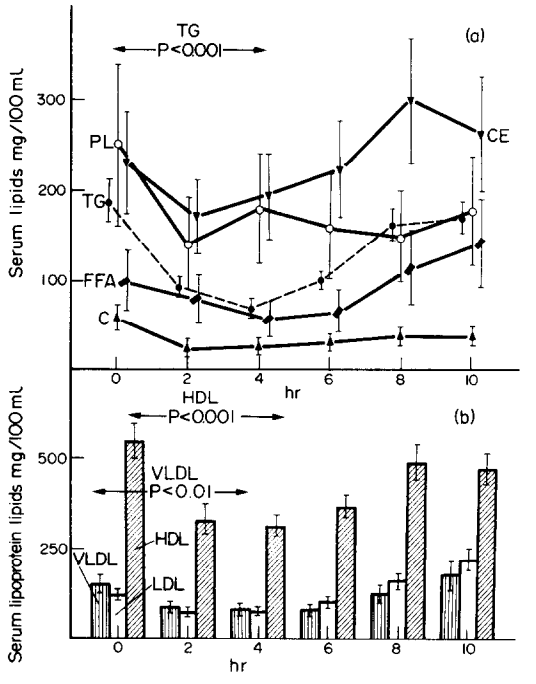

Fig. 2. The effect of 0.2 mg per kg Vincristine sulfate on the serum lipid (a) and lipoprotein (b) content of normal mice. Abbreviations: PL, phospholipids; TG, triglycerides; FFA, free fatty acids; C, free cholesterol; and CE, cholesterol esters. VLDL, very low density; LDL, low density; and HDL, high density lipoprotein fractions.

applied in a single, non-toxic dose decreased the serum lipid level. The serum lipid-lowering effect was relatively rapid and reversible. In normal animals the serum lipid level fell to minimum values within 4 to 8 hr following drug administration but returned to normal in 12 to 24 hr, approximately (Fig. 2). In the tumour-bearing animals the effect was somewhat prolonged but hyperlipidemia also recurred within 24 to 48 hr (Fig. 3). In general, the serum lipid-lowering effect of Vinca alkaloids varied from 20 to 50 per cent depending on the dose applied (Tables 1 and 2).

Among the individual serum lipid fractions first of all the concentrations of triglycerides, cholesterol esters, and to a lesser extent those of phospholipids, cholesterol and free fatty acids had a tendency to decrease. These changes were observable in both normal and tumor bearing animals, however, in the hyperlipidemic mice they were more pronounced (Fig. 3). Changes in the concentration of serum lipoprotein fractions demonstrated that under the effect of Vincristine treatment the VLDL and HDL contents decreased parallel to that of serum lipids in both normal and tumorous animals, while the LDL fractions decreased to a lesser extent or remained unchanged. Polyacrylamide gel electrophoresis

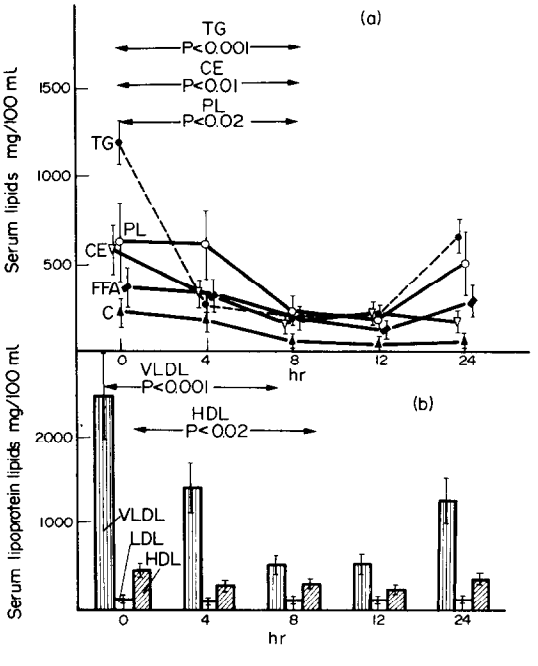

Fig. 3. The effect of 0.2 mg per kg Vincristine sulfate on the serum lipid (a) and lipoprotein (b) content of 10-day-old NK/Ly ascites tumour bearing mice. Abbreviations as in Fig. 2.

Table 1. The serum lipid-lowering effect of dimeric Vinca alkaloids in mice 4 hr after the i.p. drug administration

Alkaloids*	LD_{50} mg/kg	Doses mg/kg	Model	n	Serum lipid-lowering effect % ± SD
Vincristine	4.2	0.2	Normal	11	33.71 ± 3.05
		1.0	Normal	11	49.05 ± 3.98
		0.2	Ehrlich	11	32.02 ± 4.99
		1.0	Ehrlich	11	52.13 ± 5.72
		0.2	NK/Ly	11	38.98 ± 5.04
		1.0	NK/Ly	11	53.90 ± 9.65
Vincaleucoblastine	7.6	1.0	NK/Ly	5	47.80 ± 11.12
Vinleurosine	90.0	6.0	Ehrlich	5	49.09 ± 7.64
N-Formyl-Leurosine	29.0	3.0	Normal	11	40.40 ± 8.95
Leurosine		3.0	Ehrlich	10	60.42 ± 5.09
Penta-hydroxy-Vincaleucoblastine	> 200	10	NK/Ly	5	30.18 ± 7.40
		10	Ehrlich	5	34.59 ± 12.42
Dimethylaminoacetyl-Vincristine	> 200	10	NK/Ly	5	38.57 ± 8.65
Dimethylaminoacetyl-Vincaleucoblastine	> 200	5	Ehrlich	5	35.65 ± 4.16

* In the form of sulfates.

showed that in addition to the general decrease of serum VLDL level, Vincristine treatment evoked the appearance of VLDL fractions of lower molecular weight (Fig. 1c).

Dose dependence of the hypolipidemic effect was studied with Vincristine 4 hr after drug administration. The serum lipid-lowering effect rose in function of the dose applied and all doses examined induced qualitatively identical changes in the serum lipid and lipoprotein composition. Linear correlation was found between the decrease of serum total lipid and VLDL contents and the logarithmic values of doses applied (Table 3). Similar correlation valid for other lipoprotein fractions could not be verified. As indicated in Table 3, the decrease in the serum VLDL or total lipid content was associated with the parallel rise of total lipid and triglyceride content in the liver. The total lipid content of livers rose significantly, even under the effect of 0.1 mg per kg Vincristine as compared to the control group; however, the triglyceride content showed a marked rise only after the administration of doses over 0.2 mg per kg. The serum lipid–VLDL-lowering effect of Vincristine plotted against the doses applied yielded an exponential curve, while the lipid and triglyceride content of the liver resulted in a linear one.

DISCUSSION

The fact that the serum lipid-lowering effect of Vinca alkaloids occurs in normal as well as in various hyperlipidemic conditions of animals suggests that these compounds profoundly influence the lipid metabolism of the organism. The effects of the various monomeric and dimeric forms are qualitatively identical, but differ in quantity. The hypolipidemic effect of the monomeric alkaloids refers to the basic role of indole structure in the mechanism of action of these drugs. There is no direct correlation between the tumour-inhibitory and serum lipid-lowering effect of different Vinca alkaloids. Compounds scarcely effective or completely ineffective on tumours (e.g. monomeric forms, penta-hydroxy-Vincaleucoblastine, dimethyl-amino-acetyl-Vincristine) exert nearly the same hypolipidemic effect when applied in equitoxic doses.

One of the most characteristic features of hypolipidemic effect is the rapid and temporary decrease of serum VLDL content associated with the appearance of VLDL fractions of lower molecular weight and with the increase of neutral lipid (triglyceride) content in the liver. It is reported that Vinca alkaloids inhibit the biosynthesis of phospholipids, they slightly

Table 2. The serum lipid-lowering effect of monomeric Vinca alkaloids in mice 4 hr after the i.p. drug administration

Alkaloids*	LD_{50} mg/kg	Doses mg/kg	Model	n	Serum lipid-lowering effect % ± SD
Catharantine	> 200	20	Ehrlich	5	43.17 ± 7.57
Vindoline HCl-salt	~ 280	50	Normal	5	23.56 ± 5.11
		200	Normal	5	35.09 ± 7.07
Vindoline base†	> 200	200	Ehrlich	3	31.60 ± 15.18
Desacetyl-Vindoline	> 200	10	NK/Ly	5	22.08 ± 5.81
Vindolinol	n.d.	25	Ehrlich	5	29.42 ± 12.97
Velbanamine	> 200	3	NK/Ly	7	19.71 ± 6.74
		10	NK/Ly	5	14.06 ± 3.94
		10	Ehrlich	5	28.97 ± 17.60

* In the form of sulfates.
† Solved in carboxymethyl cellulose.
n.d. Not determined.

Table 3. Effect of Vincristine on the serum total lipid and VLDL content and on the liver total lipid and triglyceride content in Ehrlich ascites tumour bearing mice

Group VCR doses mg/kg	Serum Total lipids Q	Serum VLDL Q	Liver Total lipids	Liver Triglycerides
			mg per mg protein	
Control	—	—	0.264 ± 0.031	0.0249 ± 0.0093
0.1	0.216 ± 0.052	0.244 ± 0.039	0.332 ± 0.020*	0.0331 ± 0.0039 ns
0.2	0.300 ± 0.050	0.332 ± 0.046	0.362 ± 0.014†	0.0431 ± 0.0159 ns
0.4	0.382 ± 0.060	0.401 ± 0.052	0.397 ± 0.016‡	0.0556 ± 0.0174*
0.8	0.516 ± 0.049	0.510 ± 0.016	0.506 ± 0.077‡	0.0732 ± 0.0298†

Each group contained 5 animals. The serum lipid-VLDL lowering effect expressed as mean values of Q = total-lowered per total ± SD.
ns: Not significant, * $P < 0.02$, † $P < 0.01$, ‡ $P < 0.001$.

affect the metabolism of neutral lipids [1, 2] and they may cause a delayed hydrolysis of cholesterol esters in the liver [27]. Presumably the liver VLDL synthesis is not impaired. Electron microscopic examination of the liver of animals treated with Colchicine and Vinca alkaloids demonstrated the retention of secretory Golgi vesicles filled with VLDL particles in the liver cells [2, 10, 21, 22], suggesting that the release is inhibited, rather than the synthesis. At the same time the lipoprotein lipase system is not affected in the blood circulation; it is capable of decomposing the serum VLDL molecules to lipoproteins of lower molecular weight (intermediary density lipoproteins, remnants) and to HDL and LDL, respectively [23–25]. These processes may be responsible for the rapid fall of serum VLDL level. The phenomenon is in good agreement with the recent concept on the catabolism of low density lipoproteins of serum, except that the actual serum LDL and HDL levels may be influenced by other factors, too. For instance, the decrease in serum HDL level may be due also to the simultaneous inhibition of protein synthesis in the liver or to the decreased hepatic recirculation of HDL-apoprotein (Apo-A) caused by the inhibition of VLDL secretion [26].

The exponential dose-dependence of the serum lipid-lowering effect of Vincristine suggests that Vinca alkaloids inhibit the lipoprotein release in the liver either by blocking the active sites of membrane systems (cytoplasmic, Golgi) responsible for the secretion, and/or altering the physico-chemical properties of the membranes (e.g. fluidity). Recently, it has been shown that several amphipatic compounds including Vinca alkaloids possess marked membrane active properties [7, 8]. Further studies are needed, however, to clarify how the interrelationship of Vinca alkaloids and membrane systems of liver cells modify the transport functions of membranes.

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