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ADRIAMYCIN TREATMENT INCREASES 3-HYDROXY-3-METHYLGLUTARYL CoA REDUCTASE AND ISOPRENE BIOSYNTHESIS IN THE RAT LIVER

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Summary: Treatment of rats with Adriamycin caused an increase in the incorporation into hepatic cholesterol of [1-^{14}C] acetate, but not of [2-^{14}C] mevalonate. The step affected was found to be 3-hydroxy-3-methylglutaryl CoA reductase whose activity in the liver microsomes increased in Adriamycin-treated animals, but was inhibited when the drug was added in the assay medium. Also, the concentration of ubiquinone in the liver and of cholesterol in the plasma increased.

There is growing evidence that isoprene biosynthesis and the rate-limiting enzyme, 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, have an important role in the regulation of DNA synthesis and cell proliferation. The activity of HMG CoA reductase and cholesterol synthesis were found to be high in rapidly dividing cells (1-4). Also under conditions of induced cell division such as regenerating liver (5) or phytohemagglutin-treated lymphocytes (6), HMG CoA reductase was stimulated earlier than DNA synthesis. The loss of cholesterol "feedback control" of this enzyme during development of malignancy (5,7) also reflects the general requirement of increased activity during cell proliferation.

With the introduction of Compactin, a metabolite derived from Penicillium cultures, as a potent competitive inhibitor of HMG CoA reductase (8), new insights were obtained on the vital role of this enzyme and the product of its reaction, mevalonate, in cell division. Compactin added in the medium of cultured human fibroblasts produced large amount of HMG CoA reductase but this remained inactive in the cell in the presence of the inhibitor since biosynthesis of cholesterol from [1-^{14}C] acetate was inhibited (9). This increase in the enzyme was suppressed by adding small amounts of mevalonate but not by

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the low-density lipoproteins containing cholesterol. In synchronized cells of BHK-21, a non-malignant transformed fibroblast cell line, suppression of HMG CoA reductase activity on addition of Compactin was accompanied by prevention of the normal S-phase burst of DNA synthesis that could be restored on adding mevalonate, but not cholesterol (10). Compactin-induced abnormal embryonic development in Strongylocentrotus purpuratus appeared to be due to decreased capacity of dolichol-dependent synthesis of glycolipids and glycoproteins since normal gastrulation was restored when dolichol, a mevalonate-derived product, but not cholesterol or ubiquinone, was supplemented in the medium along with Compactin (11). In monkey arterial smooth muscle cells and Swiss 3T3 cells in culture addition of compactin inhibited the increases induced by a platelet-derived growth factor in the synthesis of cholesterol and DNA and this block in DNA synthesis was prevented when mevalonate was included along with Compactin (12).

These interesting relationships between HMG CoA reductase and mevalonate and the processes involved in cell division obtained through the use of Compactin prompted us to test the effect on isoprene biosynthesis of another drug, Adriamycin, an antracycline antibiotic from Streptomyces peucetius, effective against a variety of neoplasms (13). We report here on the increase in hepatic HMG CoA reductase and biosynthesis of cholesterol and ubiquinone in Adriamycin-treated rats.

Experimental: Male albino rats weighing 150-160 g from the Institute Central Animal Facility maintained on a pellet diet (Hindusthan Lever, Bombay) were used. Groups of six rats were administered intraperitoneally Adriamycin (0.5 mg/day per rat) as a solution in normal saline (0.1 ml) for four days. Control group was similarly injected with normal saline. The animals were killed on the fifth day under ether anaesthesia, the livers were removed and blood was drawn from the heart and collected in heparinised tubes for preparing plasma by centrifugation. Where mentioned, each animal was given intraperitoneally [1-^{14}C] acetate (10 µCi) or [2-^{14}C] mevalonate (0.5µCi), 30 min. before killing. The livers were processed for obtaining unsaponifiable lipids and fatty acids as described earlier (14). The unsaponifiable lipids were fractionated on deactivated alumina columns to separate and estimate cholesterol and ubiquinone (14,15). Hepatic microsomal fractions were isolated (16) and HMG CoA reductase was assayed by the micromethod using tlc to separate mevalonate formed (17). All biochemicals were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. [1-^{14}C] Acetate (49 mCi/mmole) and DL-[2-^{14}C]

mevalonic acid lactone (12.9 mCi/mmole) were purchased from Bhabha Atomic Research Centre, Bombay and Radiochemical Centre, Amersham, U.K., respectively. Adriamycin is a gift from Dr. M. Ghione, Farmitalia, Milan, Italy.

Results: Treatment of rats with Adriamycin resulted in an increase in the hepatic microsomal HMG CoA reductase activity by about 65% compared to the controls (Table 1). When isolated microsomes were incubated with ATP-Mg (18) similar degree of inhibition was obtained with both control and Adriamycin-treated groups suggesting that the nature of the active enzyme had not changed.

Testing the effect of Adriamycin *in vitro* on HMG CoA reductase posed some problems as the drug precipitated when added to the standard assay medium. This was traced to the phosphate buffer and glucose-6-phosphate. Therefore the activity of the enzyme in this set of experiments was measured in Tris-HCl buffer and NADPH (6mM) instead of phosphate buffer and glucose-6-phosphate plus its dehydrogenase, respectively. Since microsomes have some NADPH Oxidase activity, it was ascertained that sufficient NADPH was present in the assay system throughout the incubation period. Adriamycin when added in the assay inhibited HMG CoA reductase. Increasing inhibition was obtained with increasing concentration of Adriamycin (Fig. 1) with about 50% inhibition at 0.5 mM. From other experiments (data not given) it was found that the nature of inhibition was competitive with the substrate, HMG CoA.

As in the case of Compactin (9), Adriamycin increased the enzyme *in vivo* but inhibited it when added *in vitro*. Therefore it was of interest to test whether the enzyme, despite the increase, remained inactive in the cell as in

Table 1. Effect of Adriamycin treatment on HMG CoA reductase

Addition *in vitro*	Picomoles mevalonate/min per mg protein		%	P
	Control	Adriamycin-treated	control	value
None	109±17	180±34	165	<0.001
ATP-Mg(1mM)	57±11	95±13	167	<0.001

The values are mean ± S.D. of independent analysis of livers from six rats in each group. P values are calculated by Student's t test.

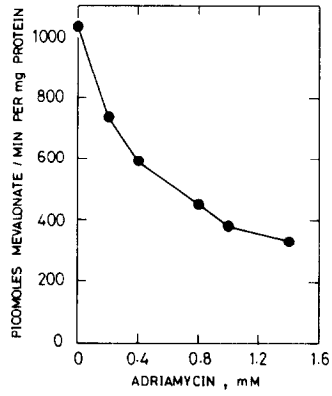

Figure 1. Effect of Adriamycin on HMG CoA reductase in vitro

the case of Compactin (9). For this purpose, [1-^{14}C] acetate was given intraperitoneally to intact animals and its incorporation into cholesterol was found to increase 4-fold in Adriamycin-treated animals compared to controls (Table 2), showing that the increased enzyme was active under the test conditions. Incorporation of [2-^{14}C] mevalonate into cholesterol, on the other hand, remained unchanged (Table 2), and this is in conformity with a change limited

Table 2. Effect of Adriamycin treatment on the biosynthesis of Cholesterol and Ubiquinone

Tracer and liver component	Counts/min per g liver		% control	P value
	Control	Adriamycin-treated		
[1-^{14}C] Acetate				
Unsaponifiable lipids	4810±1060	17010±4190	353	<0.001
Cholesterol	3740±830	15910±3070	426	<0.001
Fatty acids	22150±3680	6010±1940	27	<0.001
[2-^{14}C] Mevalonate				
Unsaponifiable lipids	19340±3130	18160±850	94	n.s.
Cholesterol	16320±2430	15980±1700	98	n.s.
Ubiquinone	440±70	750±180	170	<0.01

The values are mean ± S.D. of independent analysis of livers from six rats in each group. P values are calculated by Student's t test; n.s. not significant

Table 3. Effect of Adriamycin treatment on concentration of cholesterol and ubiquinone

	Control	Adriamycin treated	% control	P values
Liver cholesterol (mg/g liver)	2.0±0.1	2.1±0.1	105	n.s.
Plasma cholesterol (mg/100 ml)	78±3	134±8	172	<0.001
Liver ubiquinone (nmoles/g. liver)	117±18	221±23	189	<0.001

The values are mean ± S.D. of independent analysis of livers from six rats in each group. P values are calculated by Student's t test; n.s. not significant.

to HMG CoA reductase. However, the increased incorporation of [2-^{14}C] mevalonate into ubiquinone represents an additional effect of Adriamycin on the branch in the isoprene pathway leading to the synthesis of the sidechain of ubiquinone (Table 2).

The large decrease in the incorporation of [1-^{14}C] acetate into fatty acids in Adriamycin-treated animals needs special note (Table 2). It is necessary to investigate whether this represents inhibited synthesis of fatty acids and thereby of triglycerides and phospholipids or a rearrangement of flux of acetyl CoA due to the stimulated isoprene pathway.

Increased hepatic HMG CoA reductase and cholesterol synthesis in Adriamycin-treated animals had not affected the concentration of cholesterol in the liver but increased it in the plasma (Table 3), as expected, because cholesterol synthesized in the liver is known to be transferred to the blood (see 19 for a review). However, concentration of ubiquinone in the liver (Table 3) increased as a result of increased synthesis indicated above.

Discussion: There seems to be an apparent contradiction in the effects of Adriamycin on HMG CoA reductase - a competitive inhibitor *in vitro* but increases the amount of the enzyme *in vivo*. In this respect an excellent parallelism exists between Adriamycin and Compactin. But in contrast, Compactin also inhibited the increased enzyme *in vivo* and decreased synthesis of mevalon-

ate and derived products (9). It is possible that the concentration of Adriamycin in microsomes, albeit present (H. Muhammed, unpublished data), may not be high enough at the time of killing, 24 hrs after the last dose, as the drug is known to be metabolized in the liver (20). Thus the increased enzyme in both cases of drug treatment may represent compensatory response of the cell to provide itself with mevalonate-derived products, necessary for cell proliferation, and the effect on this process may be part of the mechanism of action of Adriamycin. In the light of this, it may be feasible to develop other antineoplastic drugs based on phenolic acids, known to inhibit mevalonate metabolism (21), specifically mevalonate-5-pyrophosphate decarboxylase (22).

It was reported that Adriamycin had no effect on synthesis of ubiquinone from mevalonate in beating rat-heart cells (23) and inhibited it from p-hydroxybenzoate in beef heart mitochondria (24), in contrast to our results. The increased synthesis and concentration of ubiquinone in the liver may therefore represent a specific effect in the liver tissue.

The few treatments known to increase the enzyme are: x-irradiation (25) adenosine (15), noradrenaline (26), cholestyramine (27) and Triton WR1339 (28) and during the circadian rhythm (29). Some of these responses are very rapid and offer interesting possibilities of increased synthesis as well as stabilizing the existing enzyme which need further investigation.

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