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Effects of triparanol upon the biosynthesis of cholesterol

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THE EFFECTS OF TRIPARANOL UPON THE BIOSYNTHESIS OF CHOLESTEROL

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INTRODUCTION

Considerable interest has been focused in recent years upon the possibility that an alteration of cholesterol metabolism may play an important role in atherosclerosis. The study of triparenol and its effects of lowering serum and total body cholesterol has been spurred because of the implication that vascular disease may receive beneficial effects from its action. Greater insight has also been gained regarding the process of cholesterol metabolism.

Cholesterol is a white, solid, organic chemical compound, melting point, 150° Centigrade. It belongs to the steroid group of lipids and is further classified under steroids as a sterol.

Cholesterol and other steroids are classified as lipids because they have similar solubility properties to fatty acids, though they have no obvious structural relationship.

Steroids may be considered as derivatives of a fused, reduced ring system, perhydrocyclopentanophenanthrene, comprising three six-membered rings in the nonlinear or phenanthrene arrangement, to which is condensed a five-membered ring.

In general, there is an oxygenated substituent on carbon atom three of most steroids. There are "angular" methyl groups, numbered nineteen and eighteen borne on carbon atoms ten and thirteen. There is an aliphatic substituent on carbon atom seventeen which contains eight, nine, or ten carbon atoms in the sterols, five in the bile acids, two in the adrenal cortical steroids and in progesterone, and none in the naturally occurring estrogens or androgens. Steroids are not essential in the diet, except for the vitamins D, sterol like substances. Though with radiant energy, vitamin D_3 may be formed from body synthesized 7-dehydrocholesterol.

There are nine cneters of asymmetry in cholesterol. The two of most concern are at carbon-five and carbon-ten. When

the hydrogen atom at carbon-five is on the same side of the plane of the molecule as the methyl group on carbon-ten, rings A and ' are cis to each other and the molecule belongs to the normal configuration. Rings A and B are trans to each other, and the molecule is of the alls configuration when the hydrogen atom at carbon-five is on the opposite side of the plane of the molecule from the methyl group on carbon-ten.

The hydroxyl groups on carbon-three may lie above or below the plane of the molecule. When above, the compound is of the alpha series, when below, it is of the beta series.

All steroids with the three beta-hydroxy structure are precipitated by digitonin, which itself is the glycoside of a steroid. Many analytical methods for cholesterol depend upon an initial digitonin precipitation.

Sterols have eight to ten carbon atoms in the side chain at position seventeen and an alcoholic hydroxyl group at position three. Cholesterol is the most abundant sterol in animal tissues. It also has a double bond at the five-six position.

In the blood about two thirds of the cholesterol is esterified, chiefly to unsaturated fatty acids. Reduction of the double bond at the five-six position gives rise to two products, both of which are naturally occurring, coprosterol (beta-normal) and beta-cholestanol (beta-allo). Coprasterol is the major fecal sterol. Cholestanol is a minor constituent of the sterols of blood and other tissues.

Oxidation of cholesterol yields seven-dehydrocholesterol, which has a conjugated pair of double bonds and on ultraviolet irradiation gives rise to vitamin D_3. Adrenal cortical steroids may be synthesized in vivo from either cholesterol or acetate.

Cholesterol is contained in all animal tissues. Of dietary sterols it is the only one which crosses the intestinal wall with ease and is readily absorbed via the chylous route. Its absorption is exclusively through the intestinal lasteals and depends upon the presence of bile salts in the intestinal lumen. Ferric Chloride, presumably by formation of an insoluble iron salt of bile acids, markedly reduces absorption of intestinal cholesterol.

In the absorption process the major portion of cholesterol is esterified with fatty acids. Beta-cholestanol and coprosterol are poorly absorbed from the gastrointestinal tract. Both are formed by bacterial action on cholesterol within the intestine.

There is some absorption of the plant sterol, beta-sitosterol. It appears to compete with cholesterol for "absorption sites" within the intestinal mucosa, and possibly when administered for long periods of time may effect a reduction in plasma cholesterol values.

Acetyl coenzyme A provides all the carbon atoms of endogenous cholesterol. Degradation products from fats, proteins and carbohydrates provide the acetyl coenzyme. Almost all the tissues which have been tested, including liver, adrenal cortex, and arterial wall can generate cholesterol in vitro from acetate. The enzymes involved are associated with the microsomes of the cytoplasm, but synthesis depends upon cofactors

in the surrounding fluid.

It is estimated that there are at least twenty-six reactions to go from acetylcoenzyme A to cholesterol. Two molecules of acetyl coenzyme A react to form acetoacetyl coenzyme A. Another acetyl coenzyme A molecule adds on, giving beta-hydroxyl-beta-methylglutaryl coenzyme A (HMG-coenzyme A). HMG-coenzyme A then converts to mevalonic acid, aided by another coenzyme, reduced triphosphopyridine nucleotide. Mevalonic acid goes to a pyrophosphate. Three molecules of mevalonic acid pyrophosphate combine to make farnesyl pyrophosphate. Then squalene forms from two molecules of farnesyl pyrophosphate. Squalene oxidizes to lanosterol which further oxidizes and loses three molecules of carbon dioxide to become zymosterol and then cholesterol.[1]

It is believed that there is an self-regulating mechanism by which a constant cholesterol level is maintained in body tissues. The rate at which the body synthesizes cholesterol thus varies inversely with the amount of cholesterol in the diet. Siperstein[2] believes the rate-controlling step is conversion of beta-hydroxyl-beta-methylglutaryl coenzyme A to mevalonic acid. Cholesterol feeding in the rat inhibits conversion of intermediates between acetyl coenzyme A and mevalonic acid from going to cholesterol. Cholesterol feeding does not inhibit production of fatty acids and ketones which give some of the same reactions required for HMG-coenzyme A. Therefore, Siperstein believes the inhibited reaction must be the HMG-coenzyme A to mevalonic acid step.

Other work by Siperstein suggests that with reduced glucose breakdown, ie. in diabetes, HMG-coenzyme A may build up and if other factors remain the same more cholesterol and ketones will be formed.

The more saturated the fatty acids of the diet the higher the level of serum cholesterol concentration. The elevations of blood cholesterol in nephrosis may not be due to a direct effect on synthesis, but rather to an inhibition of removal of cholesterol from the blood.

The ratio of cholesterol to phospatide remains fairly constant. Roughly two-thirds of plasma cholesterol exists esterified with fatty acids. Maintenance of this ratio is a function of the liver and the ratio of esterified to non-esterified decreases in liver disease. The liver is the chief synthetic source of cholesterol and the chief disposal agent, via the bile.

Bil'iary stasis or inflammatory disease of the gall bladder may lead to separation of cholesterol crystals from the bile and formation of bil_iary calculi. Direct excretion across the intestinal mucosa is another means of cholesterol entering the intestinal tract.

Cholesterol serves as a precourser for a variety of biologically important, structurally related steroids. Eighty per cent of cholesterol metabolized is transformed into bile acids, which are almost completely reabsorbed and return to the liver where conversion to bile salts is accomplished.

Soluble enzymes from adrenal gland, testes and ovary can

catalyze scission of the side chain of cholesterol between C-twenty and C-twenty-two, yielding pregnenolone, which may serve as a precourser of progesterone, which in turn can give rise to an array or adrenal cortical and androgenic steroid products.

The intestinal mucosa, skin and other tissues dehydrogenate a portion of cholesterol to seven-dehydrocholesterol which under the influence of ultraviolet light forms vitamin D_3.

Pathological deposition cholesterol-containing plaques in the intima of the aorta is the characteristic lesion of atheromatosis, and is seen in arterio and arteriolar sclerosis. In these conditions plasma cholesterol may not be strikingly elevated but the ratio of cholesterol to phosphatides is elevated, generally, and in centrifugal studies of the blood an increase is noted in the fraction S_f twelve to twenty, which is rich in cholesterol.

Feeding cholesterol to carnivores generally does not have pathological consequences, but atheromatosis of the aorta of the rabbit is readily produced by addition of cholesterol to the diet.

In xanthomatosis, multiple benign fatty tumors of skin, tendon sheaths and bone are seen, associated with a lipemia and a striking hypercholesterolemia.

Hypercholesterolemia is seen in hypothyroidism and nephrosis but overt deposition is not noted.

The range of normal human serum cholesterol is from 190 to 250 milligrams per 100 milliliters. This represents approximately thirty seven per cent of the total lipid content of the blood. Approximately thirty per cent of the total cholesterol is in the free state and seventy per cent is in the form of cholesterol esters. A cholesterol ester level of below fifty per cent is considered indicative of liver parenchymal damage.[3]

Only three basic methods of attempting to depress serum cholesterol have been tried. First, by attempting to decrease absorption from the gastrointestinal tract. Second, to increase the metabolic process and biliary excretion of cholesterol. Third, to inhibit the endogenous liver synthesis of cholesterol.

Unsaturated fatty acids such as linoleic acid and linolenic acid prevent absorption of cholesterol from the gastrointestinal tract, according to Keys, et al.[4]

Various authors have found diet to be of little value in reducing serum cholesterol.

Morrison[5] studied a series of 100 patients with typical, unequivocal electrocardiographic evidence of myocardial infarction and characteristic clinical history. No patient had complicating diseases such as diabetes or hypertension. Alternate patients were placed on a low calorie, low-cholesterol twenty five gram fat diet. The remainder maintained their regular diets.

Average cholesterol in the low fat diet was fifty to seventy five milligrams per day. The regular diets contained two hundred to 1800

milligrams per day. Weight loss in the first three years was noted. The average male on the low fat diet went from 166 to 145 pounds and the average woman went from 141 to 124. With the low fat diet considerable reduction in total serum lipids was noted, the mean drop being from 840 to 571 milligrams in the three years. Serum cholesterol level was the last fraction[16] drop, taking one to two years in some patients, the mean being from 312 to 220 milligrams in three years. The eight and twelve year survivors showed little change in weight or serum lipids.

After twelve years, thirty eight per cent of the patients on the low-fat diet survived and all the patients on regular diet were dead. Of both groups, most patients died of complications of atherosclerosis.

Triparanol, 1-[para-(beta-diethylaminoethoxy) phenyl]-1-(para-tolyl)-2-(para-chorophenyl)ethanol, is a white crystalline solid, melting point, 102-104° C., molecular weight, 437.98. It is soluble in alcohol, insoluble in water, and slightly soluble in olive oil.

Triparanol acts by enzymatic inhibition of liver synthesis of cholesterol.[6] The primary site of action of inhibition of cholesterol synthesis is in the conversion of twenty four-dehydrocholesterol (desmosterol) to cholesterol, which is considered to be the final step in the biosynthesis of cholesterol, occurring between zymosterol and cholesterol. Total cholesterol, cholesterol esters, and phospholipids were all depressed in serum of rats while triglyceride levels were increased. Using C^{14}-acetate, four hours after injection, specific

radio-activity of twenty four-dehydrocholesterol was approximately forty times as high as the specific radioactivity of cholesterol.

A larger amount of desmosterol accumulates in the livers of rats under triparenol administration,[7] and significant amounts of desmosterol appear in the serum of rats and humans under triparanol therapy. The Liebermann-Burchard color yield of desmosterol is fifty two per cent that of cholesterol. Zak's ferric chloride reagent gives approximately equal results with cholesterol and desmosterol. Using both the Abell and Zak methods on a mixture containing only these two compounds, the percentage of each can be calculated. On one patient with familial hypercholesterolemia, the Abell and Zak methods correlated well with chromatography in showing the cholesterol drop was replaced by desmosterol. In another patient, a thirty eight per cent drop in cholesterol was about two-fifths replaced by desmosterol.

The intermediate sterols occurring before the action of triparenol can be excreted as bile acids and unsaponifiable materials.[8] It was also found that in the rat 7.2 per cent of single doses of triparanol was excreted in the urine in forty eight hours, and 29.7 percent in the feces. In bile-fistula rats 34.5 per cent was excreted in the bile in forty eight hours.

A decrease[9] of cholesterol in several tissues of rats was found using triparanol as follows: plasma, 44 per cent; liver, 54 per cent; adrenals, 72 per cent; carcass, 28 per cent.

Gould's dosage in this experiment was fifty milligrams per kilogram.

In studying the acute toxicity of triparanol base in the rat, the LD_{50} [10] was found to be over two thousand milligrams per kilogram. The minimal effective dose was two and the maximal tolerated dose was fifty milligrams per kilogram. Other tissues showed no damage in rat, mouse, dog, and monkey, except all species showed partial lipoid depletion of the cells of the adrenal zona fasciculata.

Oaks, in a study of thirty six patients with dosages of 250,500, and 1000 milligrams, serum cholesterol depression was 69, 53, and 61 milligrams per cent respectively.[11] The greatest decline was noted during the first week. Eighty nine per cent of the subjects showed depression of serum cholesterol, but eleven did not. In a patient in whom the drug was discontinued temporarily, serum cholesterol levels began to rise, then fell again when triparanol was readministered. Of interest was the observation that dosages greater than 250 milligrams daily did not have significantly greater results.

In a continuation of the previous study[12] it was found that eighty seven per cent of the patients had a significant reduction of serum cholesterol values and over fifty per cent had over seventy five milligrams decrease. Increase was noted when therapy was discontinued and decrease was seen with reinstitution of therapy. Except for two patients, the cholesterol level remained depressed with treatment. These patients had a sudden rise in serum cholesterol followed by coronary thrombosis.

In another series,[13] eleven of fifteen patients had reduction of serum cholesterol with more than two months treatment, and four of nine with less than two months. By radioactive study,[14] the total body cholesterol was reduced forty six per cent in one patient, and in another,[15] approximately the same percentage reduction from one hundred eighty four to one hundred grams, was noted.

There apparently is no amplification of the effect of triparanol when cholesterol is removed from the diet.[16]

In some cases an escape from the action of triparanol has been suggested as serum cholesterol levels began to rise. Though some cases did not respond to increased dosage, most were seen to respond by tripling the dosage.

Fat partition studies[12] showed no correlation between changes in cholesterol levels and the triglyceride or unesterified fatty acid levels.

In a study of adrenal response under triparanol therapy seven healthy adults, and two patients with hyperadrenalism were given one to two grams of triparanol daily. In the normal subjects, two days of ACTH stimulation resulted in urinary seven-hydroxycorticoid excretion of 51.8 and 83.9 milligrams per twenty four hours before triparanol and 36.1 and 57.0 after ten days of one gram of triparanol daily. Without ACTH, daily excretion of 17-hydroxycorticosteroid dropped from 11.1 to 6.2 milligrams per twenty four hours. Seventeen-ketosteroid daily excretion was decreased from 17.1 to 12.2, but

ACTH response was 46.0 prior to triparanol and 43.0 after. Tetrahydrometabolite of aldosterone decreased from 75.1 to 56.1 daily and with ACTH from 350. to 110. Response a month after discontinuance of triparanol returned to normal.

In a patient with adrenal gland adenocarcinoma production of 17-ketosteroids decreased from 88 to 35 and 17-hydroxycorticosteroids dropped from 50 to 18 milligrams per twenty four hours.

Healthy subjects given the recommended dose of 250 milligrams of triparanol daily showed no diminution of response to ACTH.

In contrast to the previously mentioned results of depression of adrenocortical activity under doses of triparanol, another group[19] claims no change in urinary 17-ketosteroid and 17-ketogenic steroid excretion with a dosage of 250 milligrams of triparanol daily for six weeks. Plasma hydrocortisone showed no signifigant change. However, urinary 17-hydroxycorticosteroid determinations were not done so there is no comparison with the significant effect noted with the higher dosage in the previously mentioned study.

Eleven of twenty two patients with angina pectoris[20] had reproducible anginal pain and abnormal electrocardiographic changes following a given amount of exercise. During treatment three of these eleven failed to develop angina or electrocardiographic abnormalities to the same amount of exercise. After withdrawal of triparanol and substitution of placebo medication, the anginal pain and electrocardiographic changes reappeared. Iproniazid in these three patients had prevented subjective angina but had not prevented abnormal post-exercise electrocardiographic changes. Triparanol therapy had been for one to three

months and incidence of anginal attacks had decreased. A decrease in serum cholesterol had also occurred.

In another study of eighteen patients with angina,[21] six patients were classified as having a very good response and nine had no response. Of interest was the duration of angina in relation to response to triparanol therapy. Those who responded well averaged 2.9 years duration of angina, and those who did not respond averaged 5.2 years. About a patient whose cholesterol level rose while under therapy, but whose angina did not return, the author suggested that the result may not have been due to cholesterol changes but possibly due to coronary vasodilatation. However, improvement of angina is slow, rather than rapid as one would expect with a vasodilator.

Fourteen of sixteen patients with angina pectoris[22] stated that their angina disappeared within two months of therapy. Only one patient showed electrocardiographic changes, and his tracings reverted to normal. In this same series several petients volunteered that intermittant claudicatron had improved markedly; however, as this is difficult to evaluate, definite conclusions cannot be drawn.

A study of twenty six patients[23] showed no clinical or electrocardiographic responses in patients with angina given triparanol, as far as relief from angina was concerned.

Three patients under therapy with triparanol for twelve to fourteen weeks showed good serum cholesterol response but no change in eyelid xanthelasma.[24]

In renal hematologic and hepatic studies[25] of patients under triparanol therapy, bromsulphalien, cephalin flocculation, prothrombin time, SGOT, SGPT, nonprotein nitrogen, PSP urinalysis and complete

blood count studies were done as well as cholesterol and cholesterol ester ceterminations. One patients cephalin-cholesterol went from one plus to four plus and back to two plus under therapy and another patient's SGOT rose from three to forty two, then decreased to thirty three. These were the only laboratory value changes. No interferance with cholesterol esterification was noted.

No effect on blood clotting mechanism[26] was noted in patients under anticoagulant therapy and in normal patients both groups being treated with triparanol or placebo.

Other effects noted in a few patients under triparanol therapy were nausea and rashes,[27] both of which were transient in patients continued on therapy, or when dosage was reduced.[11] Also noted were false positive urinary albumin tests.[28]

In the literature, several authors were quite enthusiastic about the therapeutic and research possibilities of triparanol but it was often stated that it would take several years to adequately evaluate the drug.

SUMMARY

Cholesterol is a steroid compound classified as a lipid because of solubility properties similar to fatty acids. It is found in all animal tissues. The sources of body cholesterol are diet and synthesis in many body tissues, including liver, adrenal cortex, and arterial wall. Acetyl coenzyme A provides all the carbon atoms of endogenous cholesterol. Cholesterol is a precourser of several steroids within the body. In atherosclerosis, fatty plaques of high cholesterol content are found in arterial walls, serum cholesterol may or may not be elevated, however. Normal serum cholesterol is between 150 and 250 milligrams per 100 milliliters, approximately two-thirds of which is esterified.

Considerable work has been done in attempting to reduce serum cholesterol and attempting to determine whether or not a beneficial effect upon the process of atherosclerosis may be obtained.

Among the methods suggested are decreasing absorption, increasing the metabolic process and excretion, and inhibiting the endogerous secretion of cholesterol. The latter effect is that of triparanol, 1-[para-(beta-diethylaminoethoxy) phenyl] 1-(para-tolyl)-2-(para-chlorophenyl) ethanol. Its site of action is between desmosterol and cholesterol, the last stage of cholesterol synthesis.

Several studies have noted a signifigant decrease in serum cholesterol under triparanol therapy. Typical results were those of Oake who found 89 per cent of his patients responded to triparanol therapy

most in one week, with the average decrease in one month ranging from 53 to 69 milligrams per cent with groups on 250, 500, and 1000 milligrams daily. The now recommended dosage is 250 milligrams daily. Cholesterol levels tend to rise with discontinuation of therapy and fall again when therapy is resumed. A few patients have been noted to "escape" from triparanol control but increased dosages usually resume control. Intermediate sterols can be excreted as bile acids, though some rise of desmosterol has been noted. By the Zak method cholesterol and desmosterol giveequal results, but by the Liebermann-Burchard method, the color yield of desmosterol is fifty two per cent that of cholesterol.

Triparanol reduces the cholesterol content of many tissues, and in two patients the total body cholesterol was reduced 46 per cent.

With high dosages, 1000 milligrams daily, it has been found that corticosteroid production, daily and under stress, is reduced, both in normal patients and in patients with adrenal tumors.

Adrenal suppression with the usual dose has not been reported. There are characteristic microscopic changes of adrenal tissue, however.

In patients with angina and electrocardiographic changes with the Masters test, there are reports of beneficial effects, decrease of angina and reversal of electrocardiographic findings. These changes were seen mainly in patients with angina for two to four years. Those with longer history of angina did not respond well. Other authors report no beneficial effects on angina.

No change of eyelid xanthelasma was found.

No significant laboratory changes were noted in renal, hematologic, and hepatic studies to indicate damage to these organs.

Toxicity of triparanol is low. Side effects in a few patients included nausea, rashes, and false positive urinary albumin.

CONCLUSION

Triparanol has been shown to decrease serum cholesterol in a significant number of patients by inhibiting the synthesis of cholesterol. The site of action is believed to be between the desmosterol and the cholesterol, the last step in cholesterol biosynthesis. Total body cholesterol is thus decreased. In the study of triparanol, important contributions have been made to the knowledge of the process of cholesterol biosynthesis. Several years study will be needed before it can be determined whether or not there is a beneficial effect upon the cardiovascular system or if there are any harmful effects under prolonged triparanol therapy.

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