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Review Article

OXIDATIVE STATUS AND ORAL CONTRACEPTIVE. ITS RELEVANCE TO PLATELET ABNORMALITIES AND CARDIOVASCULAR RISK

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Abstract—Oral contraceptive (OC) use is a risk for thrombogenic events. This paper reviews effects of OC on oxidative status, coagulation, and platelet activity. Complicating effects of cardiovascular risk factors such as smoking, diabetes, hyperpidemia, and hypertension, are discussed. From these data we conclude that:

1. OC use modifies slightly but significantly the oxidative status in women and in animals by decreasing in plasma and blood cells the antioxidant defenses (vitamins and enzymes).
2. The changes in the oxidative status are related to an increase in plasma lipid peroxides apparently responsible for the hyperaggregability and possibly the imbalance in clotting factors associated with the OC-induced prethrombotic state.
3. These effects of OC appear to be increased by a high intake of polyunsaturated fat and counteracted by supplements of vitamin E.
4. The risk factors acting synergistically with OC, have all been shown to increase platelet reactivity. In addition, smoking, diabetes, and, to some extent, dyslipidemia are associated with an increased level of lipid peroxides and concomitant changes in the antioxidant defenses that can be additive to those induced by OC. Thus, free radicals and lipid peroxidation could be the underlying mechanism in the predisposition to thrombosis induced by most risk factors in OC users.
5. Results of epidemiologic and experimental studies in this field will be concordant only when diet and natural antioxidants will be systematically taken into consideration.

Keywords—Free radical, Lipid peroxidation, Hormonal contraceptive, Thrombosis, Platelets, Vitamin E, Vitamin C, β-carotene, Glutathione

INTRODUCTION

Hormones administered orally to prevent contraception have been used for the last 30 years in women. Although early in this century, precursors have shown that estrogenic tissue extracts could inhibit pregnancy as reviewed by Goldzieher,[1] the hormonal contraceptives were devised following the discovery by Gregory Pincus that oral progestational 19-nor steroids could inhibit ovulation.[2] The idea behind was to reproduce artificially a state of pregnancy in order to prevent all possibilities of fecundation. For that aim, Pincus used substances close to progesterone but active at low dosage and after oral administration.[2,3] Following their success in female rat and rabbit, the studies were conducted in women.[2,4] When a synthetic estrogen was added to the progestative substance to improve its clinical tolerance,[5] the hormonal contraceptive was born.

At present, throughout the world, several million women use the pill as it is now known. Hormonal contraceptives certainly constitute the most effective reversible method for birth control. Their pharmacological and pathological effects have been studied extensively over the years. In an attempt to decrease their toxicity, changes in posology have been introduced with time up to the minipills that are used nowadays.

As a matter of fact, as early as 1961, Jordan reported a case of pulmonary emboli attributed to the pill.[6] In 1962, the pill was associated with stroke.[7] In subsequent years, the administration of hormonal contraceptives was reported to be a risk factor for several vascular

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problems.[8–11] With the use of minipills and the identification of the associated risk factors, the risks have been steadily decreasing since these early reports. Nevertheless, it seems that even today there is a certain risk associated with that kind of contraception. Our studies suggest that the main noxious effect of the pill could be an alteration of the oxidative status. Data available so far will be examined in light of this concept.

THE CARDIOVASCULAR RISK OF ORAL CONTRACEPTIVES (OC) IN 1990

In the 1970's, the increased mortality from cardiovascular disease with the use of OC was established by several studies, the most extensive being that of the Royal College of General Practitioners'',[9] the Oxford Family Planning Association,[10] and the Kaiser Permanente Walnut Creek Prospective Contraceptive Study.[11] The three main vascular problems linked with the use of combined hormonal contraceptives (OC) were venous thromboembolism, thrombotic stroke, and myocardial infarction, that is, coronary thrombosis.[12] However, the reported data have been criticized extensively as summarized by Shearman in 1981.[13] It has been emphasized that OC predispose

- only to certain forms of cardiovascular disease,
- in women aged 35 years or more while it is mostly young women that use the pill.

Obviously, cardiovascular disorders present a multifactorial etiology, a favorable change in one risk factor being probably able to counteract an unfavorable change in another. Finally, over the years, there have been many changes in the pill formulation and in the way it has been used.

In more recent studies on 65,000 healthy women,[14] it has been shown that there was a positive association between the pill and venous thromboembolism with a rate ratio of 8.3 for the follow-up from 1977 to 1979, and 2.8 from 1980 through 1982. For both periods, there was no association between OC use and stroke or myocardial infarction.[15] Two subsequent studies have been even more reassuring concerning the occurrence of fatal conditions in healthy OC users. In 50,000 woman-years of observation among women aged 15–44 who use the pill from 1977 to 1982, there was no significant evidence of OC use on mortality.[16] In general, the authors seem to agree that the decrease in the association between OC and cardiovascular disorders in USA is due to

- the decrease in the dosage of estrogen in OC;
- the selection of the healthiest women to receive OC;
- the use of OC before the age of 35 years.

In a review of the more recent epidemiologic data,[17] Mishell concluded that there was an increase in the incidence of cardiovascular disease and mortality only in OC users older than 35 and who smoked. The use of the pill containing less than 50 μg estrogen does not appear to be associated with an increased risk of cardiovascular disease in healthy, nonsmoking women, even 35 to 45 years of age[17] in USA, with the diet presently used.

However, it seems that even in recent years the combined smoking and OC use contributes substantially to the excess cases of myocardial infarction among US women aged 35 to 44,[18] the risk of increasing synergistically when these behaviors are combined. An extensive review of the epidemiologic data available concluded that the proposition that OC cause cardiovascular diseases was not proved scientifically[19] because of possible biases in the studies. However, *a randomized trial without possible criticisms, large enough to assess the occurrence of rare cardiovascular events in young women, is simply not feasible.*[19] Consequently, prudent medical practice requires taking into account that a risk of cardiovascular events still exists, especially in populations with borderline deficient diets. It is confirmed by recent case reports of thrombotic phenomena in unusual locations such as visceral arteries[20] and retina,[21] also attributed to OC. Thus, a deeper knowledge of the possible mechanisms involved in the noxious side effects of OC seems needed to be able to prevent completely these rare, but frequently dramatic, thrombotic manifestations that are associated with the pill.

OXIDATIVE STATUS AND HORMONAL CONTRACEPTIVES. SIMILARITIES WITH PREGNANCY

The oxidative status of an organism can be defined as the balance between free radical generation and destruction by the natural defense system as reviewed by Machlin and Bendich.[22] A molecule containing one or more unpaired electrons can be designated a free radical.[22] These unstable molecular species induce tissue damage by reacting primarily with polyunsaturated free fatty acids in cellular membranes, but also with sulfur-containing enzymes and proteins. However, free radical formation is needed for many essential biochemical reactions in the organism. Cell oxidases and electron transport system are continuous sources of free radicals.

Cells are usually efficient to scavenge free radicals. As reviewed by Niki,[23] cells contain two categories of protective systems: the preventive and the chain-braking antioxidants. Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase but also, substances

such as albumin, ceruloplasmin, and β-carotene reduce the generation of free radicals and are considered preventive antioxidants. The chain-breaking antioxidants act by scavenging free radicals and thereby blocking the oxidation by molecular oxygen. These chain-breaking antioxidants are divided into

1. water soluble, such as ascorbic acid, cysteine, glutathione;
2. lipid-soluble, essentially vitamin E.

The amount of free radicals that can be damaging to an organism under specific conditions appears to be difficult to evaluate. A more practical approach is to determine in blood and cells the level of antioxidants.

The first antioxidant found to be decreased in tissue of animals receiving estrogens was vitamin C, as reported in 1936 by Mosonyi (cited by Kalesh et al,[24]). Later on, the observation was extended to plasma in estrogen-treated guinea pigs.[25] It was further shown in monkeys that OC increases the dietary requirement for vitamin C.[26] The decrease in vitamin C pursuant to OC administration in women was confirmed in most of the studies in plasma and platelets,[24] urine[27,28] and leukocytes.[29] However, a recent study in women who smoked and were on OC indicated that the plasma level of ascorbic acid was influenced by the smoking habit but not by the OC, at the dosage presently used.[30]

Concerning vitamin E, the main lipid-soluble antioxidant, several studies have reported a decreased level of this vitamin in plasma,[31,32,33] even with the low-dose estrogen contraceptive.[33] However, the vitamin E decrease was not observed in all studies.[34] There are at least two explanations for the discrepancies between studies:

1. OC, especially the progestative fraction,[35] are known to change the lipoprotein pattern.[36] Since vitamin E in plasma is carried by lipoproteins[37] especially HDL,[38] the indirect effect on vitamin E via lipoproteins is probably variable depending on the pill. One possibility of solving this problem is to determine vitamin E in platelets rather than in plasma, as shown by Lehman et al.[39]
2. The level of vitamin E in plasma is also influenced by the intake of this and other vitamins, depending on the dietary habits usually not evaluated in such studies. To eliminate these different pitfalls, each woman should be used as her own control by comparing the level of vitamin E at day 5 and day 21 of the menstrual cycle.[40] Under these conditions, the large interindividual variability in the plasma level of vitamin E is eliminated and in OC users, a small but consistent fall in this vitamin in plasma is observed.[40] The same remark also applies to vitamin C level*. In women not receiving OC, the plasma level of vitamins E and C at day 21 of the cycle is not changed or even slightly increased.* Therefore, it seems that in women using OC, there is a significant decrease in levels of vitamin E and C, suggesting an increase in the activity of the oxidative phenomena.

As already mentioned, a factor markedly affecting the level of the antioxidant vitamins is the dietary habits of the subjects. In rats given ethynyloestradiol at half the dosage blocking ovulation, a significant decrease was observed in the plasma level of vitamin E on a saturated fat-rich diet.[41] Moreover, in the animals given a polyunsaturated (n-6)-rich diet, the estrogen induced a much more drastic decrease in the level of vitamin E despite a similar intake in this vitamin associated with an increased response of platelets to aggregation.[41] In women, also, it seems that the increased response of platelet aggregation induced by OC is potentiated by a high intake of n-6 polyunsaturated fatty acids.[40] Therefore, the need of antioxidant vitamins both in animals and women is amplified by OC or at least by the estrogen moiety, especially on a polyunsaturated-rich diet requiring additional antioxidant protection. In rats and women, these phenomena can be counteracted by a supplement in vitamin E that increases vitamin E in both plasma and platelets.[40,42] This supplementation could be required in all women using OC.

As to the preventive antioxidants, β-carotene, a singlet oxygen quencher, has been found to be depressed in plasma by OC[34] a result that we have confirmed recently.* The lower level of β-carotene appears to be associated with an increased level of vitamin A, observed in several studies.[34,43,44] It has been speculated that the increased level of vitamin A was resulting from an increased conversion of β-carotene.[34] Whatever is the mechanism, an increase in plasma level of vitamin A does not add to its antioxidant capacity since vitamin A does not have such a property, but rather requires further antioxidant to preserve its structure. As emphasized by Gey and Colleagues, "prevention effects of all essential antioxidants may require an optimal status of every single one."[45]

Another substance, ceruloplasmin, which oxidizes ferrous ions to less reactive ferric ions and binds copper, is also considered a preventive antioxidant.[23] Its level has been shown to be increased by the pill in several studies,[26,46,47] a result which could be considered

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beneficial. Nevertheless, the level of copper runs parallel to that of ceruloplasmin in OC users[46] as well as in pregnancy but also, in infection and inflammatory diseases.[48] Moreover, the levels of copper and ceruloplasmin are inversely related to that of ascorbic acid in monkeys treated with OC.[26] Since it has been shown that ceruloplasmin catalyzes the oxidation of ascorbic acid in vitro,[49] ceruloplasmin could be responsible for the increased requirement of ascorbic acid in OC users.[34] Further studies seem to be required to determine whether, at least with OC administration, ceruloplasmin plays the role of a preventive antioxidant. Possibly, also in relation to the level of ceruloplasmin, serum iron has been reported to be increased in OC users,[50] and could also be implicated in the oxidative process.[51]

As to the enzymes that reduce the generation of free radicals, an increase in the erythrocyte glutathione peroxidase activity has been found in long-term OC users.[52] An increase in this enzyme activity could represent a protective response of erythrocytes to the reported increased level in blood of hydrogen peroxides and lipoperoxides in OC users.[52,53] Under these conditions, the increase in glutathione peroxidase activity was associated with a lower riboflavin status[54] that had also been described by certain[55,56] but not all[57] previous investigators measuring the vitamin-dependent enzyme glutathione reductase. The decrease in the activity of this enzyme indicates riboflavin deficiency. It has been postulated that the decreased glutathione reductase is the result of increased glutathione peroxidase activity. The reductase is needed to regenerate the reduced glutathione from the oxidized product of glutathione peroxidase activity. Reduced glutathione per se exhibits antioxidant activity.[55]

The direct evidence of a change in the oxidative status is the observation that the blood level of lipid peroxides is increased, a result that has been reported in women receiving OC.[52,53,58] We have confirmed this result in female rats given OC[59] and shown that the peroxidized free fatty acids formed under these conditions were directly responsible for the increased platelet aggregability induced by OC.[59] The noxious effect on platelets of the peroxidized fatty acids could be blocked by various antioxidants such as glutathione, vitamin E, and catalase.[59] Thus, it seems that in both women and female rats treated by OC, a change in the oxidative status can be demonstrated, not only by changes in the level of various natural antioxidants, but also directly by observing an increased level of lipid peroxides. In addition, these lipid peroxides can modify directly the platelet reactivity probably involved in the predisposition to thrombosis, observed in OC users.

In pregnancy, similar changes in the oxidative status to those observed with OC administration, have been reported. As in OC users, an increase in serum lipid peroxide level combined with a marginal riboflavin deficiency, particularly at the late stage, has been described.[60,61,62] In pregnant rats, the increased serum lipid peroxide level was confirmed but was not found to be associated with a decrease in the plasma level of vitamin E,[63] contrary to what was observed in rats treated with estrogens.[42] By contrast, the level of vitamin E in liver and lung was decreased in pregnants rats.[63] In addition, a vitamin E deficient diet induced in pregnant rats the production of a much greater amount of lipoperoxides.[63] The activity of antioxidant enzymes catalase and glutathione peroxidase declined in the liver of pregnant rats[63] as in plasma and erythrocytes of pregnant women.[64] Thus, both in OC users and in pregnancy, the oxidative status is changed by the increased production of lipid peroxides and an increased requirement of antioxidant vitamins such as vitamin E. It was known for years that a deficiency in vitamin E was associated with fatal thrombotic (eclamptic) disease in pregnant rats.[65]

Both pregnancy and OC administration seem to affect the plasma level of other vitamins such as ascorbic acid, riboflavin, and β-carotene. In ongoing studies in OC users, we have observed that by giving a supplement in vitamin E (200 mg daily), the level of vitamin C comes back to the normal, but that of β-carotene remains low, and should probably also be corrected by a supplementation.

As a result of the increased level of lipid peroxides the activity of antioxidant enzymes is affected as shown by the decrease in the activity of catalase and glutathione peroxidase in pregnancy and the increase in glutathione peroxidase in OC users. The apparent discrepant results between OC and pregnancy concerning glutathione peroxidase have not been elucidated yet but could represent different stages of a similar stimulation induced by lipid peroxides in blood or of the intensity of this stimulation, accompanied by more profound hormonal and metabolic changes in pregnancy.

Therefore, it seems that both in pregnancy and in OC use, direct (increase in blood lipid peroxides) as well as indirect (changes in the level of antioxidant vitamins and the activity of antioxidant enzymes) evidence indicates that the oxidative status is unsettled. These modifications are probably the result of an increase in the level of estrogens (above usual physiological values), known to be associated with free radical generation.[66]

HORMONAL CONTRACEPTIVES, HYPERCOAGULABILITY, AND PLATELET HYPERAGGREGABILITY

Extensive review articles are available on the subjects but they all reflect the conflicting results obtained

in this field.[67,68] The confusion starts with the term hypercoagulability used either to qualify a disseminated intravascular coagulation, a prethrombotic state or an acceleration of the clotting process in vitro. A thrombotic event is much more complex than simply the clotting process of blood in vivo.

Thrombosis needs a triggering event, probably endothelium injury or substances such as bacterial endotoxins, epinephrine,[69] and probably many others, able to initiate platelet aggregation and fibrin formation at least as shown in animals. Once the event is triggered, the outcome probably depends on several other factors such as vortices, stasis, and the balance between the factors promoting platelet aggregation, adhesion, clotting, and those inhibiting the process such as prostacyclin, antithrombin, and fibrinolytic activity. Hypercoagulability could be defined as the acceleration of the clotting process shown by laboratory tests just as hyperaggregability is an increase in the in vitro response of platelets to aggregation. These tests can eventually characterize a prethrombotic state provided they have been shown to predict thrombosis. This implies that the relationship between the laboratory tests and thrombotic events has been established, preferably in animal studies to begin with, an approach that is most of the time totally neglected. Then the results have to be confirmed in humans, necessarily in prospective studies since once the thrombotic process is initiated, drastic changes occur in platelet behavior and clotting activity. Owing to the relative rarity of thromboembolic events in OC users such a prospective study is not feasible. By contrast, experimental data indicate that both the response of platelets to aggregation[69,70,71] and the clotting activity of platelets [72,73] evaluated before triggering thrombosis, are perfectly correlated[73] with the severity of the thrombotic events in rats fed different fats. Concordant with those experimental results, it has been shown recently that spontaneous platelet aggregation in vitro was able to predict coronary events and mortality over a 5-year period.[74] In addition, in a prospective study on more than 2000 subjects in Wales,[75] the response of platelets to aggregation by ADP and thrombin was significantly related to the prevalence of myocardial infarction. Data in relation to the incidence will be available soon. If confirmed, these data demonstrate that platelet hyperaggregability indicates a prethrombotic state. Although these studies will not yet prove a causal relationship, the success of aspirin in decreasing coronary events demonstrates that platelet reactivity is closely related to coronary thrombosis.[76] Of course, these results do not demonstrate that platelet reactivity is responsible for the thrombotic events observed in OC users. However, since in OC users, predisposition to both coronary and venous thrombosis has been reported, a similar mechanism can be suspected, substantiated by the in vitro and animal studies described hereafter. The same applies to clotting tests since it has been reported that factor VII and fibrinogen were stronger predictors than cholesterol of ischaemic events in man.[77]

OC AND CLOTTING FACTORS

In OC users, most of the clotting factors such as fibrinogen, factors VII, IX, X, XII have been reported to be elevated.[67,68] Nevertheless, all these factors are normally largely in excess as already emphasized[67] and, consequently, an increase in the activity of one or several of these factors does not necessarily results in the acceleration of fibrin formation. This is shown by studies in which blood is carefully collected in siliconized material with slow-speed centrifugation to remove most of the platelets without damage. Under these conditions, the plasma clotting time of platelet-poor plasma with[78] or without[79] the addition of cephalin, was not shorter (accelerated) either in OC users or in pregnancy. It was shorter when platelets were activated in some way either by high-speed centrifugation of the blood, or by collecting blood in nonsiliconized material.[79] The demonstration that the shorter clotting time of platelet-poor plasma in OC users was due to material released from platelets was done by running on the same samples a Stypven test, specific or at least highly susceptible to platelet phospholipids. Since the results of the Stypven time were absolutely parallel to those of the recalcified platelet-poor plasma, this indicated that the recalcified clotting test was in fact evaluating the activity of the platelet phospholipids as further shown in subsequent studies.[80] The two implications of these results are:

1. the hypercoagulability observed in pregnancy and in OC users is due to the clotting activity of platelets as previously observed with saturated fat diets[72,73];
2. the accelerated clotting tests of the plasmatic clotting factors observed by some investigators but not all[67] seems due to the experimental conditions, that is, the extent of phospholipids released from platelets during storing and preparation of the samples. This applies, in fact, to any determination evaluating the activity of a clotting factor through a clotting test as opposed to chemical determination. The clotting tests are certainly specific in order to evaluate a state of deficiency but do not appear specific enough in cases of hypercoagulability.

The clotting activity of platelets has been called platelet factor 3. It is the only clotting factor of lipid origin (phospholipids)[81] constituted by components of the platelet membrane,[82] a surface needed for the activation of the plasmatic clotting factors. In OC users, the increased activity observed could be due to an enhanced

surface availability or to the increase in the activity of the platelet phospholipids. By disrupting totally the platelet membrane by sonication, the totality of the activity of platelet becomes available.[80,81] Under these conditions, the clotting activity of OC users is still observed, suggesting an increase in the activity per se of the phospholipids.[79] The enhanced activity of the platelet phospholipids do not appear to be due to changes in fatty acid composition[83] comparable to those observed in animals[73] or in human[84] on different dietary fats. A substance such as lanosterol that has its biosynthesis increased in animals receiving OC,[85] is able to increase the activity of the platelet membrane[86] observed in OC users.[79] Nevertheless, although lanosterol biosynthesis is increased in women on the pill,[87] we have not been able to demonstrate that the level of lanosterol was enhanced significantly in platelets of OC users.[87] Another possibility for the increase in platelet clotting activity is lipid peroxidation as shown clearly for the response of platelets to thrombin-induced aggregation[59] after OC treatment, since platelet clotting activity and aggregation to thrombin are usually closely correlated.[84] In addition, it has been shown that sulfhydryl oxidation leads to activation of the platelet-clotting activity, by exposing phosphatidylserine,[85] the phospholipid the most active on clotting.[81] This phospholipid on the membrane surface increases more than a million fold the rate of thrombin formation.[89] However, the definite relationship between the increase in platelet clotting activity and peroxidation in OC users, has not been clearly demonstrated yet.

As to fibrinogen, its concentration has been found increased in many studies on OC users.[68] The higher level seems to depend on the dose of the estrogen,[90,68] although it has been found even with the minipill,[91] at least in the first 2 months of administration. A more recent study found an increased level of fibrinogen only in obese OC users.[92] However, in this last study as in many others, they did not look at smoking as a possible confounding factor. Still, it has been shown in man that much of the association between smoking and coronary events appeared to be mediated through fibrinogen.[77,93] These results are of special interest for OC users since smoking combined with OC use has been associated with a 7.2 risk of myocardial infarction.[94] Fibrinogen is also known to be raised in response to a number of stimuli such as trauma, infection, and inflammatory process in general, known to stimulate the production of free radicals.[95]

Thus, the increased level of fibrinogen observed in OC users, could also be associated with the production of free radicals, responsible for the increase in lipid peroxides.[52,53,58,59] As to the role of an enhanced level of fibrinogen to predispose to thrombosis, possible mechanisms have been reviewed recently.[96] The effect that seems to be the most feasible could be through an increase in platelet aggregability to ADP[97] lately associated with myocardial infarction.[75]

In the process of clotting, the formation of thrombin from prothrombin is inhibited by natural anticoagulants, mostly antithrombin III, which also inhibit most of the clotting enzymes generated during clotting. Antithrombin activity has been shown to be decreased in OC users both in serum and plasma.[98] More recent results indicate that with lower estrogen content of OC, there is still a true decrease of antithrombin III in plasma.[99,100] Of interest is that lipid peroxides have been shown to reduce the thrombin-neutralizing activity of plasma and the activity of purified antithrombin III.[101] Again, at another level of the cascade conducive to a thrombotic process, lipid peroxides are implicated and could be responsible for the predisposition to thrombosis of OC users. Similarly, protein S, a cofactor for the anticoagulant effect of activated protein C, has been found significantly decreased in both pregnancy and OC users as well as in inflammation.[102] Thus, a relationship between lipid peroxides and a decrease in the anticoagulant activity of protein C can be suspected and needs to be investigated.

OC AND PLATELET AGGREGATION

Contrary to what has been frequently emphasized,[67] the technique of platelet aggregation conduces to highly reproducible results[71,75] in the same individual, provided the technique is carefully standardized[103] as any other technique, including the selection of appropriate equipment. Under these conditions, the results of platelet aggregation are even internally consistent as shown by the close relationship in the response to different agonists. For instance, on 2000 subjects in Wales, the correlation coefficient for the response to thrombin vs ADP was $r=0.48$, and for ADP versus collagen, $r=0.43$.

The interest of this evaluation is that, in recent studies, an increased response of platelets (hyperaggregability) by this technique has been clearly associated with coronary thrombosis (myocardial infarction) in man[74,75] as it was associated with the severity of venous thrombosis in animals.[69,71]

In OC users, an increase in the response of platelets to aggregation has been reported in some[104,105] but not all studies.[106] In the earlier work of our group,[78] we found that platelet aggregation to thrombin and ADP was increased significantly in pregnant rats but not in pregnant women. In OC users, platelet aggregation was slightly increased as compared to controls but owing to a large interindividual variability, the difference was not statistically significant.[78] In more recent studies,[87] identical results were found. However, when the results were expressed as the percentage of abnormal values (hyperaggregable subjects), only 13% of these abnormal val-

ues were in the controls, while 43% were in the group with OC. Subsequently, by comparing platelet aggregation at day 5 and day 21 of the cycle in OC users, part of the interindividual variability was eliminated since the subjects were their own control. Under these conditions, at least the secondary aggregation to ADP, was significantly more elevated at day 21 after OC administration.[40] Our most recent results* indicate that even with the minipill, at day 21 as compared to day 5 of the cycle, there is an increase in the response of platelets to aggregation significant mostly for thrombin and secondary aggregation to ADP. By contrast, in age-matched controls with similar dietary habits, there is no increase in platelet reactivity at day 21 of the menstrual cycle.

In female rats, a highly significant increased in platelet aggregation is already observed 4 days after OC administration.[83,107] Moreover, we have shown that this response as well as that of the lipid biosynthesis, is due to the estrogen moiety,[107,108] depends on the intake of polyunsaturated fat,[41] and could be counteracted by the concomitant administration of vitamin E.[42]

In women also,[40] we observed that the increased aggregability was promoted by the intake of polyunsaturated fat this effect being eliminated by the administration of a modest supplement (200mg daily) of vitamin E.

These results seem to explain the discrepancies between the studies and the interindividual variability, probably due largely to the dietary habits of the subjects, especially the intake of polyunsaturated fat and natural antioxidants. They also suggest that the enhanced platelet reactivity could be due to the increased lipid peroxides known to occur in OC treatment.[52,53,58] This hypothesis has been confirmed at least in female rats given OC.[59] Normal rat platelets incubated only 4 min in a platelet-poor plasma of OC-treated rats, become highly hyperaggregable mostly to thrombin, even after being washed. This hyperaggregability can be blocked by either pretreating the platelets with BHT, or the plasma with antioxidant enzymes such as glutathione, peroxidase, or catalase. We found that it was the peroxidized free fatty acids that were responsible in plasma for transforming normal into hyperaggregable platelets, both to thrombin and ADP.

As to the mechanism involved in the increased response of platelets induced by lipid peroxides, it is apparently not through an enhanced biosynthesis of other lipid fractions since the effect of lipid peroxides is rapid (with 4 min) while the lipid biosynthesis is increased after incubation of 60 min.[59] For explaining such a rapid effect of peroxides it has to be a direct effect on the membrane, possibly on the glycoproteins acting as receptors since it has been shown that contraceptives increase the level of sialic acid (consequently of glycoproteins), in the platelet membrane[109] as well as the fibrinogen binding.[110] However, further work in that connection is certainly needed.

Another untoward consequence of the increased lipid peroxides as shown by Warso and Lands,[111] is the possible resulting unbalance between thromboxane (TXA_2) and prostacyclin (PGI_2).[112] It had already been reported that this balance was altered in OC users,[112] platelet responsiveness to TXA_2 being enhanced, and to PGI_2 decreased.[113] Other investigators found only a decrease in antiaggregatory PGI_2[114] and others no alteration at all in this balance.[115] However, as mentioned for the response of platelets to aggregation, production of thromboxane, one step in the total process of platelet aggregation, depends largely on the dietary habits of the subjects. It is probably the same for PGI_2 production. Consequently, it can be expected that studies performed in different countries[112–115] (USA, Norway, Finland, Germany) with different dietary habits, lead to different or even opposite results. Thus, to obtain concordant data in this field, further basic work should be performed using different diets, or at least with a careful control of the dietary habits.

In conclusion, platelet aggregability, which has been related recently to myocardial infarction, seems to be increased in OC users. At least in animals, the hyperaggregability observed with OC is due to the estrogen, induced by the resulting increase in lipid peroxides, and modulated by the intake of polyunsaturated fat and antioxidants.

HORMONAL CONTRACEPTIVES AND OTHER RISK FACTORS

Epidemiologic studies have shown that the risk for OC users to develop cardiovascular thrombotic events was increased considerably when the subjects were smoking,[9,11,17,18,116,117] had diabetes,[118] dyslipidemia,[119] or hypertension.[117,118]

For instance, in U.S. nurses using OC, the risk of myocardial infarction was three times higher but was 170 times higher if in addition they smoked and were hypertensive.[117] In Great Britain, in comparison to women not having risk factors, those with three or more factors (OC, dyslipidemia, hypertension, smoking, diabetes) had a relative risk of 78 to 1 to develop myocardial infarction. The conclusion of these studies was that the combined effect of the risk factors was clearly synergistic. Consequently, it seems of interest to examine the possible mechanisms involved in the effect of other risk factors in OC users, and to determine to what extent lipid peroxides can contribute to their additive or synergistic effects.

OC AND SMOKING

Numerous studies[9,11,17,18,116,117] seem to be unanimous in that smoking is one of the main risk factor for

cardiovascular events in OC users, especially myocardial infarction. An explanation could be that the mechanism of their untoward effects is somewhat similar. Smoking seems to predispose mainly to thrombosis like the use of OC, rather than increasing the severity of atherosclerosis. This is suggested by the observations that:

- Smoking increases markedly the risk of myocardial infarction (coronary thrombosis) in men[120,121] and women[122] but weakly, if at all, that of stable angina pectoris[123] related directly to the severity of atherosclerotic lesions.
- There is still a very high association between smoking and myocardial infarction after adjusting for coronary occlusions visualized by angiography.[124]
- When men[120] and women[122] stop smoking, the risk drops rapidly suggesting that the mechanism involved is not mostly on atherogenesis.

Thus, a direct effect of smoking on thrombogenesis can be postulated. It is substantiated by a rapid effect (within 5 min) on platelets to increase by 40 to 100% their aggregation induced by different agonists, and to increase their clotting activity.[125,126] This effect that apparently lasts as long as the subject is smoking every 30–40 min (i.e., most of the day in heavy smokers), is additive to the effect of the diet.[127] Unfortunately, to our knowledge, it has not been determined yet whether the potentiating effect of smoking on platelet reactivity is additive or synergistic to that induced by OC.

As to the factor in cigarette smoke that is responsible for the increased platelet reactivity, it has not been totally elucidated yet, although nicotine in vitro reproduces most of the effects of a cigarette at the level of blood nicotine induced by that cigarette.[125,126] Nevertheless, the level of tar seems related even more closely to the observed effect on platelets.[125] Of interest in that connection is that Church and Pryor have shown that each puff of a cigarette contains 10^{14} free radicals in the gas, and 10^{15} in the tar phase.[128] Consequently, further studies are needed to determine whether it is not the free radicals from the tar phase that are mostly potentiating the effect of smoking on platelet reactivity. This hypothesis is substantiated by the fact that plasma indices of lipid peroxidation are increased and natural antioxidants decreased in cigarette smokers.[129] This lower level of antioxidants in smokers has been observed for vitamin C [30,130] and for vitamin E in the alveolar fluid,[131] a result concordant with the increased susceptibility to peroxidation of erythrocytes from smokers.[129] This last effect was prevented by supplementation with vitamin E. Finally, the intake of β-carotene as well as its level in plasma, is decreased in smokers.[132] In other terms, smoking seems to have identical effects on the oxidative status to those observed in OC users. It seems logical that they potentiate each others effect on thrombogenesis.

OC AND DIABETES

Diabetes is a well-known risk factor for coronary heart disease.[133,134] It is also known that the risk of cardiovascular mortality depending on the severity of diabetes is much higher in women (from 2.9 to 7.3) than in men, (1.7 to 2.7)[133] and, as already mentioned, markedly increases the risk of myocardial infarction in OC users.[118,119] Concerning the mechanisms through which diabetes and OC administration exhibit potentiating effects, OC per se have been shown to be diabetogenic.[135] In addition, even low dose OC may lead to a decrease in peripheral insulin sensitivity in diabetes.[136] As reviewed recently,[137] part of the diabetogenic process in humans is apparently mediated by white cell production of active oxygen species. Thus, it is conceivable that the lipid peroxides known to occur in OC users[52,53,59] could participate in the diabetogenic process. Moreover, an increased production of lipid peroxides has been reported in poorly controlled type I and type II diabetes,[138] that could add their effects to those produced by OC administration.

As in OC treatment, diabetes is associated with changes in the oxidative defense systems. In that connection, the role of vitamin E abnormalities in diabetes-induced platelet hyperactivity has been reviewed extensively recently.[139] The level of vitamin E in platelets and liver has been shown to be depressed in rats with streptozotocin-induced diabetes.[140] Similarly, in patients with non insulin-dependent diabetes, platelet vitamin E was decreased and related to the hyperaggregability induced by ADP and to increased thromboxane production.[141] In addition, in type I diabetes, a negative correlation has been found between the synthesis of thromboxane and the level of vitamin E in plasma and platelets.[142] By contrast, an increase in plasma and platelet vitamin E has been reported in type II diabetes.[143] The discrepancies are probably the result of differences in the treatment and possibly also, as in OC use, of the dietary habits. What seems clear is that in Type I diabetes, platelet hyperactivity has been normalized by vitamin E supplementation in two independent double-blind controlled studies.[144,145] Concerning vitamin C, it seems to be decreased in plasma of both human and animal diabetics.[146,147]

In type II diabetes, the level of glutathione seems to be decreased in erythrocytes[148,149] while no consistent changes were observed in glutathione peroxidase activity.[137] Conflicting results have also been reported for other antioxidants and peroxidase.[137]

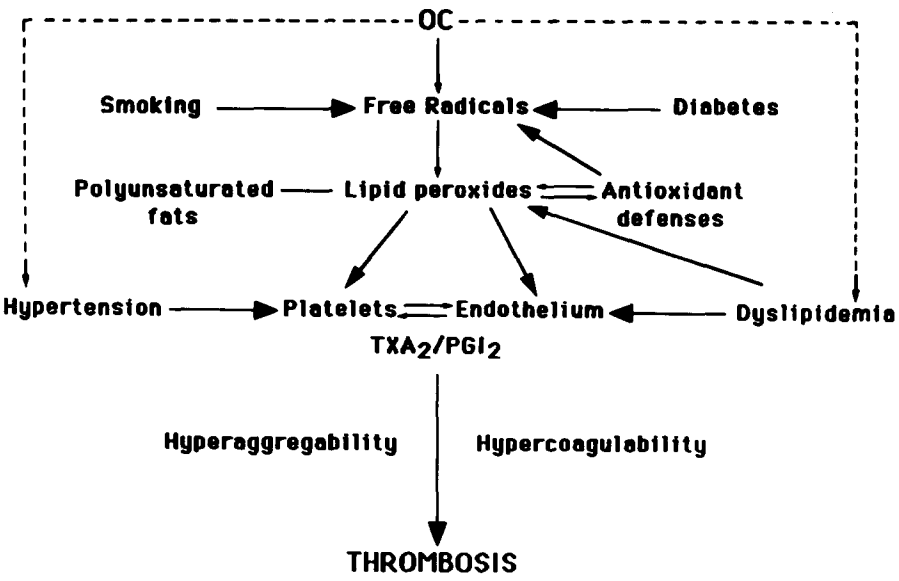

Fig. 1. Possible mechanisms involved in the OC induced predisposition to thrombosis.

Further studies seem to be needed concerning the oxidative defenses in experimental and human diabetes with an adequate control of the diet which, as in OC users, could be responsible for the contradictory results reported. Nevertheless, the production of lipid peroxides seems to be the possible mechanism for the increase in platelet aggregability documented to occur in diabetes.[134,150] However, as for smoking, it remains to be determined whether the effect of OC administration on platelets is additive to that induced by diabetes.

OC AND DYSLIPIDEMIA

The relationship between dyslipidemia and coronary heart disease has been extensively reviewed and does not need to be covered again here. It seems sufficient to mention that also in OC users, dyslipidemia seems to be a risk factor for myocardial infarction.[119] This is not unexpected since OC have been shown for years to decrease the serum level of HDL-cholesterol (HDL-C)[151,152] known to be inversely associated with myocardial infarction, especially in women,[153] even after adjusting for other risk factors. In the context of thrombosis and lipid peroxidation, the mechanism of a possible protective effect of HDL-C has not been documented yet although its level has been related postively to the intake of vitamin C[154] in women. In addition, HDL have been shown to regulate PGI_2 activity.[155]

Concerning the low density lipoproteins, peroxidation has been shown to be involved in their atherogenicity.[156] Recent studies also indicate that subjects with high LDL and low HDL cholesterol have an increase in plasma and erythrocyte lipid peroxides.[157] The connection between LDL and lipid peroxides could explain the stimulating effect of LDL on thromboxane formation by human platelets.[158] This result is concordant with the increased response of platelets to ADP and epinephrine observed in type II (high cholesterol, high triglycerides),[159] type IIA (high cholesterol and LDL)[160] and confirmed in type IIB patients (high cholesterol, triglycerides, LDL, and VLDL)[161] especially secondary aggregation to ADP, thromboxane dependent. Nevertheless, in these studies, the patients were on a high polyunsaturated fat diet, thus prone to lipid peroxidation. Consequently, it is difficult to attribute the platelet hyperaggregability to the hyperlipoproteinemia per se or to the diet since, by increasing for 1 year the intake of polyunsaturated fat from 4.8 to 8.9% of calories in normal subjects, secondary aggregation to ADP was increased significantly.[162] Moreover, in healthy farmers from France and Great Britain[84] as well as in the 2000 subjects studied in Wales,[75] serum cholesterol, HDL, or triglycerides were not correlated with any test of platelet reactivity including ADP-induced aggregation. Consequently, it does not seem to be settled yet whether it is hyperlipoproteinemia per se that influences platelet reactivity, or whether it is the associated diet.

Contrary to common belief, a high intake of linoleic acid, possibly by increasing susceptibility to peroxidation, does not seem to prevent coronary heart disease.[163] Studies on 9000 subjects have shown that in primary prevention a P/S (polyunsaturated/saturated fatty acids) ratio of 1.6 versus 0.3 does not reduce myocardial infarction and total mortality, especially in woman.[164] In addition, a recent study in patients with coronary-bypass surgery[165] has shown that the highest

dietary risk to develop new coronary lesions within 2 years was a high intake of polyunsaturated fat (above 8% calories). The efficient P/S ratio to prevent coronary heart disease seems to be lower than 0.7 as reviewed recently.[166]

OC AND HYPERTENSION

Hypertension seems to increase markedly the risk of myocardial infarction in OC users.[111,112] Possible mechanisms for the combined effects include the increase of hypertension by OC[167] and platelet hyperaggregability already observed in spontaneously hypertensive rats[168] and in essential hypertension in humans.[169] However, to our knowledge, lipid peroxidation has not yet been shown to be a mechanism in the potentiating effect of hypertension in OC users, although it has been reported recently that the urinary excretion of thromboxane B_2 was significantly higher in pregnancy-induced hypertension.[170]

Consequently, concerning the risk factors associated with the predisposition to thrombosis in OC users, the production of free radicals in tobacco smoking and diabetes could be additive to that induced by OC and result in further untoward changes of the oxidative status. Such a mechanism has not been demonstrated yet in dyslipidemia and hypertension, although it can be suspected in dyslipidemia. At any rate, these two conditions are known to induce platelet hyperactivity, probably additive to that induced by OC.

CONCLUSIONS

The possible effects of OC to promote a prethrombotic state when combined with other risk factors is summarized in Fig. 1. Whatever the adverse effects of OC, they have apparently been markedly decreased by lowering the hormone dosage in the pill. Nevertheless, even at the present dosage, estrogens generate free radicals.[66] Thus, OC administration unbalances the oxidative status that is further deteriorated by the addition of risk factors that also induce the production of free radicals and promote lipid peroxidation. In situations where a deficiency in the oxidative status can be suspected (smoking, diabetes, diet insufficiency . . .), an evaluation of the antioxidant nutritional status as defined by Gey[45] should be prescribed before the use of OC. Determination of platelet reactivity, probably the key factor in the whole thrombotic process, could be performed routinely. Another conceivable preventive measure is the addition, directly to the pill, of vitamin E that has been shown to improve the oxidative status in OC users as well as platelet reactivity. Finally, since estrogenic and carcinogenic properties have been apparently separated in estrogens,[171] further work should be urgently performed to try to separate the estrogenic from the free radical-inducing properties of OC now that a sizeable part of the thrombogenic mechanisms has been unraveled and associated with lipid peroxidation.

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