N,N′-Diacetyl-L-cystine (DiNAC), the Disulphide Dimer of N-Acetylcysteine, Inhibits Atherosclerosis in WHHL Rabbits: Evidence for Immunomodulatory Agents as a New Approach to Prevent Atherosclerosis

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ABSTRACT
Oxidation of lipoprotein-derived lipids is generally accepted to be important in atherogenesis, and lipophilic antioxidants have been suggested as potential antiatherosclerotic agents. The antiatherogenic effects observed by certain antioxidants, especially probucol, in different animal models support this suggestion. There are however also cases where other lipophilic antioxidants have not been able to support this hypothesis. This has raised the question whether the effects of probucol and similar compounds are mainly due to some other property, unrelated to their antioxidant efficacy. For example, probucol is shown to possess immunomodulatory properties. Immune reactions are known to occur during atherogenesis. We therefore tested the dimer of N-acetylcysteine, DiNAC, which is a disulfide with immunomodulating properties and enhances oxazolone-induced contact sensitivity (CS) reactions in mice, for effects on atherosclerosis. When given to male heritable hyperlipidemic rabbit (WHHL) rabbits from 10 to 22 weeks of age, this compound reduced by 50% thoracic aorta atherosclerosis (p < 0.05), without affecting plasma lipid levels. Here we also show that probucol and a close chemical analog, both known to prevent atherosclerosis in WHHL rabbits, enhance the CS reaction in mice, while two other related antioxidants did not affect the CS reaction. At least one of these is also without effect on atherosclerosis in WHHL rabbits. The results show that DiNAC might represent a new treatment modality for atherosclerosis-related disease, and suggest that some antioxidants may have antiatherosclerotic properties more related to “immunomodulatory” properties than to antioxidant properties in general.

According to the LDL oxidation hypothesis, LDL-derived oxidized lipids assist in foam cell formation, are cytotoxic, and instigate various proatherogenic processes (Steinberg et al., 1989). Increasing antioxidant defense has therefore been proposed as a therapeutic way to reduce atherogenesis (Steinberg et al., 1989). The original experimental support for this was that the antioxidant probucol was antiatherosclerotic in heritable hyperlipidemic rabbits (WHHL rabbit) (Carew et al., 1987; Kita et al., 1987). Following these observations, antiatherosclerotic effects of other lipophilic antioxidants have been demonstrated in several species, but there are also several studies with a negative outcome (Pettersson et al., 2000).

The LDL oxidation hypothesis is hampered by a lack of correlation between ex vivo measures of antioxidant effects and antiatherosclerotic effects of drugs obtained in vivo (Fruebis et al., 1994, 1995, 1997; Witting et al., 1999b; Djahansouzi et al., 2001). The major metabolite of probucol found in rabbits is a bisphenol compound (H 212/43; Table 1), which in itself is an antioxidant. In WHHL rabbits, a virtually complete inhibition of the accumulation of oxidized lipids (measured as cholesteryl ester hydroperoxides and hydroxides) was obtained by H 212/43 in the aorta, but no inhibition of formation of atherosclerotic lesions. Probucol administration also prevented lipid oxidation (probably due to high concentrations of the metabolite H 212/43), along with the expected antiatherosclerotic effect (Witting et al., 1999b). This finding thus showed dissociation between the antiatherosclerotic and antioxidant properties of H 212/43. In mice, probucol reduced atherosclerosis without inhibiting lipid peroxidation (Witting et al., 2000)

Immune mechanisms operating in the arterial wall are important for atherogenesis, although the nature of these

ABBREVIATIONS: LDL, low-density lipoprotein; WHHL, heritable hyperlipidemic rabbit; DiNAC, N,N′-diacetyl-L-cystine; CS, contact sensitivity reaction; DTH, delayed type hypersensitivity reaction; TBARS, thiobarbituric acid-reactive substance; NAC, N-acetylcysteine; Asc, ascorbate.
reactions is not clarified in detail (Hansson, 1997; Ross, 1999; Lusis, 2000). Numerous experiments, especially in genetically engineered mice, show that manipulations of various components of the immune system can dramatically affect lesion formation (Roselaar et al., 1995; Dansky et al., 1997; Daugherty et al., 1997; Boring et al., 1998; Mach et al., 1998; Gosling et al., 1999). Hyperlipidemia per se may also affect the immune responses in atherosclerosis by altering the balance between Th1 and Th2 type reactions of the immune system (Zhou et al., 1998). These findings indicate that proinflammatory reactions are part of the pathophysiology of atherosclerosis, and compounds modifying immune responses may thus be of interest as potential antiatherosclerotic drugs. Experience in rabbits and mice indicate that a gross reduction of cell-mediated immunity by cyclosporine A treatment can in fact enhance atherosclerosis (Emeson and Shen, 1993; Roselaar et al., 1995). Thus, it is unclear which properties of an immunomodulatory compound prevent the development of atherosclerosis. In mice, the cholesterol lowering agents statins appear to have antiatherosclerotic effects unrelated to cholesterol-lowering activity, but associated to an anti-inflammatory effect (Sparrow et al., 2001), further supporting that inflammatory reactions in the artery wall is important in atherogenesis.

\( N,N'\)-Diacetyl-l-cystine (DiNAC), the disulfide dimer of \( N\)-acetylcysteine, is a potent modulator of contact sensitivity (CS) and delayed type hypersensitivity reactions (DTH) in rodents, probably acting by interference with the immune system (Särnstrand et al., 1999). Interestingly, it differently affected CS/DTH responses depending on whether the agents used to elicit the responses induced Th1- or Th2-type reactions. The major aim of this study was to investigate whether DiNAC has antiatherosclerotic properties. In the initial studies of the relation between chemical structure and efficacy, it was suggested that an intact disulfide bridge was required for the effect on the oxazolone-induced CS reaction (Särn-
strand et al., 1999). Probucol has two sulfur atoms in proximity (Table 1) and can affect certain functions of the immune system (Ku et al., 1988, 1990; Akeson et al., 1991; Zapolska-Downar et al., 2001). A second aim was to compare the effects of probucol and some closely related chemical analogs on the CS reaction, and compare them to those of DiNAC. The results show that DiNAC, which is not an antioxidant, reduced lesion formation in WHHL rabbits to an extent similar to that of probucol. Furthermore, probucol and a close chemical analog, both known to prevent atherosclerosis in WHHL rabbits (Carew et al., 1987; Kita et al., 1987; Mao et al., 1991), enhanced oxazolone-induced CS reaction in mice similar to DiNAC. In contrast, the two other probucol analogs did not affect the CS reaction, although they possess antioxidant activity. The one of these latter that was examined for antiatherosclerotic effects failed to express such activity.

Materials and Methods

Chemicals. 4-Ethoxy-methylene-2-phenyl-2-oxazolin-5-one (oxazolone) was from either BDH (Poole, Dorset, England) or from Sigma (St. Louis, MO). Probucol was purchased from Jucker Pharma (Stockholm, Sweden), and H 212/43 from Polysciences Inc. (Warrington, PA). DiNAC lysine salt was synthesized at AstraZeneca Process R&D (Södertälje, Sweden). H 330/47 and MDL 29,311 were synthesized at AstraZeneca R&D Malmö (Malmö, Sweden).

Antioxidant Potency. We used two methods to compare antioxidant efficacy of the compounds studied. First, oxidation potential was determined using cyclic voltammetry. Typically, one milligram of the compound was dissolved in 3.4 ml of 99.5% ethanol, whereafter 0.6 ml of a 0.2 M solution of ammonium acetate was added. The voltammograms were recorded using a Voltamograph CV-27, Cellstand C1B, using a glassy carbon electrode, a Ag/AgCl reference electrode, and an auxiliary Pt electrode (Bioanalytical Systems, West Lafayette, IN). The antioxidant property of the compounds was then tested in a Fe-ascorbate-mediated lipid peroxidation assay using sonicated soybean phospholipid suspension as described previously (Westerlund et al., 1996). Thiobarbituric acid reactive substances (TBARS) formed during oxidation was evaluated using a fluorometer (Westerlund et al., 1996). Thiobarbituric acid reactive substances were enzymatically determined in plasma using commercially available kits (Roche Molecular Biochemicals, Mannheim, Germany).

Evaluation of lesion volume was performed as described (Witting et al., 1999b). Briefly, animals were anesthetized, the chest opened, the heart exposed and perfused (−1.5 liters) with Dulbecco’s phosphate-buffered saline, followed by fixation with formal saline (1.5 liters), and the thoracic section was removed. Approximately 10 cross sections (each 2 μm in thickness, 1 mm apart) were cut from paraffinized sections of aorta centered around the first pair of intercostal artery branches. They were stained with Weigert’s hematoxylin-van Gieson, and quantitation of cross-sectional areas of intima and media was obtained by planimetry, using a Lucivid device (MicroBrightField, Colchester, VT) attached to a Leitz DRM microscope. This device superposed a computer-generated display onto the microscope image. The external and internal elastic laminae were highlighted using the mouse-operated cursor and the media cross-sectional area obtained as the difference between the two areas enclosed. All intimae present were considered as atherosclerosis and their cross-sectional areas obtained as the area confined by the internal elastic laminae minus the lumen area. Finally, each area was converted to a volume by multiplying by 1 mm (the distance between two sections), and such volumes were determined over all the sections and expressed as a total volume over the segment investigated. Mean cross-sectional area of both the media and intima were also calculated for each individual. Planimetry was performed using Microvid Software (MikroMakro AB, Gothenburg, Sweden) in a blinded manner using coded samples. Aortic volume is shown as the intima-to-media fractional volume; such normalization for differences in the size of the aorta specimens reduced variability between animals.

Statistics. For mouse experiments, the results are expressed as the mean ± S.E.M from groups of 8 to 10 animals. Degree of significance for differences between means of groups was obtained by Student’s two-tailed t test. In the rabbit experiments, all results are expressed as mean ± S.E.M. Effects of treatment were evaluated by Student’s t test for unpaired observations. The distribution of the volume measures for atherosclerosis follows a lognormal distribution, so the comparisons were made after log transformation.
Results

The compounds investigated for their effect on the CS reaction in mice in the present study are shown in Table 1. The table also shows that DiNAC, which is the oxidized form of NAC, is not an antioxidant in the two simple tests used to describe antioxidant potency. In the cyclic voltammetry test, DiNAC was not oxidized at the highest voltage used (1 V), and its capacity to inhibit TBARS formation in the Fe/Asc system was poor, with an IC_{50} above 10^{-5} M. In contrast, the antioxidants used were all oxidized in the voltammetry test, and the IC_{50} obtained for inhibition of the Fe/Asc system were all at or below 10^{-6.5} M.

The CS reaction experiments were performed in four separate experiments, with results obtained summarized in Fig. 1. All drugs used were well tolerated. In the first experiment, which was not performed in a blinded manner, we found that both probucol and MDL 29,311 significantly enhanced oxazolone-induced CS. Probucol may have a bell-shaped dose-effect relation, as the highest dose used reversed the response, although the effect of the highest dose was still significantly higher than that of the vehicle. In the second experiment, we directly compared the effects of DI-NAC and probucol on the CS reaction, and found that both drugs enhanced the reaction over a wide range of doses. We were thus able to repeat the results previously obtained with both DI-NAC (Särnestrand et al., 1999) and probucol (experiment 1).

We then went on to investigate whether the effect of probucol and MDL 29,311 is common to all antioxidants. First, we examined how the bisphenol probucol metabolite H 212/43 affected CS. This compound, which contains no sulfur atoms and is not antiatherosclerotic in WHHL rabbits (Witting et al., 1999b), did not affect CS reactions in mice in the doses investigated (Fig. 1, experiment 2). H 330/43 is also an antioxidant probucol analog (Table 1), and it has one sulfur atom. Two doses of this compound also failed to affect the oxazolone-induced CS reaction in mice (Fig. 1, experiment 4). This compound has not been investigated for its effect on atherosclerosis.

The antiatherosclerotic effect of probucol is not necessarily related to its antioxidant properties (Witting et al., 1999b, 2000). Therefore, we decided to test whether an agent that is not an antioxidant, but expresses an effect on the CS reaction similar to probucol, can inhibit atherosclerosis. We examined the effect of DI-NAC on atherosclerotic lesion formation in WHHL rabbits. The rabbits were administered 0, 0.03, or 3 μmol of DI-NAC/kg of b.wt./day through the drinking water, doses known to have intermediate and optimal effects on oxazolone-induced CS reaction in mice (Fig. 1, experiment 2), from age 10 weeks until termination at 22 weeks of age.

For the analysis of aortic lesions, we used the same measure of intima volume in the thoracic aorta as described previously (Witting et al., 1999b). After perfusion fixation, we removed the thoracic aorta and cleaned it from adventitial fat. Approximately 1-cm-long segments centered around the first pair of intercostal arteries were used for lesion volume determinations. Mean cross-sectional areas of the media were 2.82 ± 0.13 mm² in the controls, 2.78 ± 0.19 mm² in the low, and 2.79 ± 0.17 mm² in the high DI-NAC groups, respectively. Intima mean cross-sectional areas were 0.84 ± 0.23 mm² in the controls, 0.59 ± 0.07 mm² in the low, and 0.37 ± 0.09 mm² in the high DI-NAC groups, respectively. The resulting intima/media ratios from the volume determinations are summarized in Fig. 2. DI-NAC caused a dose-dependent inhibition of lesion development. The largest effect was found for the high dose, which is the dose causing maximal stimulation of the oxazolone-induced CS reaction (Fig. 1; Särnestrand et al., 1999), while the lower dose appeared to be of intermediate efficacy. The intima/media ratio in the high dose group was smaller than that in the controls (p < 0.5), whereas the effect of the low dose did not reach statistical significance. Because there was no change in the dimensions of the media, the treatment effect appeared to be solely due to a reduction in intima growth. Three micromoles of DI-NAC/kg of b.wt./day caused a reduction in lesion volume comparable with that of 1% probucol in the diet (from 30–15% of media volume in this study, compared with a reduction from 34–15% with probucol) (Witting et al., 1999b).

Blood samples taken before onset of treatment and at termination showed that total serum cholesterol and triglyceride concentrations were not affected by the drug treatment (Table 2).

![Fig. 1. Effects of treatment with DiNAC and antioxidants on the CS reaction in mice. The compounds used and the doses administered (in μmol/kg of b.wt./day) in the experiments are indicated at the base of each column. Four different series of experiments are shown, except for experiment 1 they were conducted in a blinded manner. The results are shown as mean ± S.E.M.; an * indicates that the response is significantly different from the corresponding control experiment (p < 0.05). n = 8 to 10 animals/group. The results clearly show that probucol, MDL 29,311, and DI-NAC enhance oxazolone-induced CS in mice, while H 212/43 and H 330/43 are ineffective.](image-url)
Fig. 2. Lesion volume was estimated in approximately 1-cm-long segments of the descending thoracic aorta and normalized for the size of the vessel segment. Drug treatment with DiNAC appeared to cause a dose-dependent reduction in lesion volume; for the highest dose, this was a statistically significant change. Doses are micromoles per kilogram of body weight per day.

TABLE 2
Effects of DiNAC on plasma cholesterol and triglyceride concentrations
Blood samples were taken before (0 weeks) and after 12 weeks of treatment with DiNAC in the three groups. The table shows the mean values ± S.E.M. of cholesterol and triglyceride concentrations in plasma. n = 10 (controls and 3 μmol/kg of b.wt./day) or 9 (0.03 μmol/kg of b.wt./day).

<table>
<thead>
<tr>
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<th>Cholesterol</th>
<th>Triglycerides</th>
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<tr>
<td></td>
<td>controls</td>
<td>0.03 μmol</td>
</tr>
<tr>
<td></td>
<td>0 Weeks</td>
<td>12 Weeks</td>
</tr>
<tr>
<td>Control</td>
<td>11.2 ± 0.65</td>
<td>11.5 ± 0.75</td>
</tr>
<tr>
<td>0.03 μmol</td>
<td>10.1 ± 0.68</td>
<td>12.7 ± 0.49</td>
</tr>
<tr>
<td>3.0 μmol</td>
<td>10.2 ± 0.89</td>
<td>10.4 ± 1.29</td>
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Discussion

This study provides the first evidence that DiNAC, a compound known for its immunomodulating activity, has antiatherosclerotic effects in WHHL rabbits. DiNAC may thus represent a new treatment principle for atherosclerosis-related diseases. The rationale for examining this compound for effect on the development of atherosclerosis included the observations that the antiatherosclerotic effect of probucol cannot be easily explained by its antioxidant properties (Fruebis et al., 1997; Witting et al., 1999b, 2000), that probucol is capable of enhancing T-cell-mediated immunity in a simple in vivo model in a manner similar to DiNAC (Fig. 1), and that probucol also affects other inflammatory reactions. Because plasma concentrations of cholesterol and triglycerides were not affected by DiNAC treatment (Table 2), we conclude that the antiatherosclerotic effect of the treatment was not due to an effect on lipid metabolism.

In the mouse, both 0.03 and 3 μmol of DiNAC/kg of b.wt./day significantly enhanced the CS reaction (Fig. 1). Only the higher dose of DiNAC reduced lesion development significantly, the lower dose did not statistically significantly reduce lesion development (Fig. 2). This discrepancy can be due to species differences in the sensitivity to DiNAC. However, atherosclerosis measures have normally larger coefficients of variation than we obtained in the CS experiments, so this difference between the effects on atherosclerosis and CS reactions may simply be due to too low number of rabbits in the atherosclerosis experiments.

Table 3 is a schematic summary of the experiments performed. It shows that there is a clear positive association between enhancement of the CS reaction and an antiatherosclerotic effect among the compounds tested, but that antioxidant properties do not seem to predict for an antiatherosclerotic effect. Except for probucol, we tested three chemically related compounds (Table 1) for their effects on the CS reaction. MDL 29,311 contains two sulfurs (as probucol does) and is shown to reduce lesion development in WHHL rabbits (Mao et al., 1991). H 212/43 is an effective antioxidant that contains no sulfur atom and does not affect WHHL rabbit lesion formation (Witting et al., 1999b). H 330/43 was chosen because it is an effective antioxidant containing a single sulfur atom. MDL 29,311 affected the CS response similar to probucol and DiNAC, while the other two compounds were ineffective in these experiments. Together, these results further support the hypothesis that the antiatherosclerotic effect of probucol (and MDL 29,311) can be due to an immunomodulatory, rather than antioxidant, property of the compound. Previous studies have reported that probucol can affect cytokine production and adhesion molecule expression in macrophages (Ku et al., 1988, 1990; Ake-son et al., 1991; Zapolska-Downar et al., 2001). Thus, the finding that probucol can act as an "immunomodulator" is not entirely new, but here we provide collected evidence that this effect is not mediated through its antioxidant properties. A number of other structurally unrelated antioxidants has previously been investigated for their effects on atherosclerosis, with highly variable outcome (for review, see Pettersson et al., 2000). Except for the present study, little is known on whether these antioxidants of different chemical classes differ with respect to their immunomodulatory properties, and whether this can contribute to explain the discrepancies between different antioxidants.

The antioxidants used in the present study were characterized only by some in vitro antioxidant properties; in vivo measures of antioxidant capacity were not investigated. Depending on the oxidizing conditions, the efficacy of these lipophilic antioxidants in preventing lipid peroxidation will be different (Upston et al., 1999). Table 3 also shows some in vivo antioxidant properties of the drugs used (when known). Probucol likely prevents against in vivo lipid peroxidation in rabbits by conversion to its metabolite H 212/43, because probucol did not prevent against lipid peroxidation in mice.

Table 3
Summary of the known effects of the compounds used on atherosclerosis in WHHL rabbits, the CS reaction in mice, and their antioxidant properties

<table>
<thead>
<tr>
<th></th>
<th>Atherosclerosis</th>
<th>CS/DTH</th>
<th>Antiox in Vitro</th>
<th>Antiox in Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>DiNAC</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>n.a.</td>
</tr>
<tr>
<td>Probucol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MDL 29,311</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
</tr>
<tr>
<td>H 212/43</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H 330/43</td>
<td>n.a.</td>
<td>−</td>
<td>+</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

n.a., not analyzed.
where the metabolite H 212/43 was found in only very low concentrations (Witting et al., 2000). Because there is no definite agreement between in vitro and in vivo antioxidant effects, it would be interesting to know whether, e.g., DiNAC possesses antioxidant properties in vivo, in spite of it not being an antioxidant in vitro. Little is known about its metabolism, but it could, for example, be metabolized to NAC, which is an antioxidant. However, the dose-effect relationship observed for DiNAC and NAC in the CS system strongly argues against this possibility (Särnstrand et al., 1999). Although this remains to be investigated, the pattern is that prevention of oxidation of lipoprotein-derived lipids in general will not explain the antiatherosclerotic properties of the compounds investigated.

We have previously suggested that an intact disulfide bridge was required for DiNAC and its chemical analogs to be effective as immunomodulators (Särnstrand et al., 1999). The present experiments indicate that this might not be fully true because probucol and ML 29,311 both have a carbon atom separating the two sulfurs. Because neither H 330/43 nor H 212/43 have two sulfurs (Table 1), it appears that at least two sulfur atoms have to be in proximity to allow for the effects of these compounds on the CS reaction. Alternatively, some but not all antioxidants may have this immunomodulatory property, but that this effect is not specifically dependent on the presence or absence of sulfur.

There are some limitations to the present study. First, we used different animal models for the CS reactions and atherosclerosis experiments. The mouse is the species normally used for studies on CS/DTH reactions. DiNAC is shown to enhance the CS reaction also in rabbits (Särnstrand et al., 1999), but probucol and its analogs have not been tested for the effects on CS or DTH responses in this species. We chose WHHL rabbits to assess the effects of DiNAC on atherogenesis because this model was the one used to provide the original experimental support for the LDL oxidation hypothesis (Carew et al., 1987; Kita et al., 1987). Also, the effects of antioxidants on atherosclerosis in mice may differ from those in other species. H 212/43 does not inhibit atherosclerosis in rabbits (Witting et al., 1999b) but is reported to do so in mice (Witting et al., 1999a). Even probucol may differ between species, although the proatherosclerotic effect described in mice may rather be a site-specific phenomenon limited to atherogenesis in certain parts in the aortic root (Pettersson et al., 2000; Witting et al., 2000). Thus, there might be species differences with respect to effects of antioxidants on lesion formation. Further studies to investigate this matter are ongoing, and preliminary data show that DiNAC has an antiatherosclerotic effect also in mice (Westin Eriksson and Pettersson, 2000).

Second, a direct comparison between the effects of DiNAC and probucol (and the other compounds used) on atherosclerosis was not performed. With the limited availability of WHHL rabbits, we choose to use two doses of DiNAC instead of including probucol as a positive reference. However, in a previous study in which we used the same protocol (Witting et al., 1999b), probucol reduced lesion volume by 56%. In the present study, the highest dose of DiNAC resulted in a reduction of lesion size with 50%, so the effect seems comparable. Direct comparisons of these compounds are ongoing.

Third, and most importantly, the above-mentioned argumentation is by association rather than through a cause-effect relationship. There is today no knowledge on the mode of action by which DiNAC and probucol exert their purported immunomodulatory effects resulting in a reduced lesion formation. Preliminary results do however show that DiNAC can reverse endothelial dysfunction caused by atherosclerosis and/or hypercholesterolemia (Brandt-Eliasson et al., 2000). This is also known for probucol (Anderson et al., 1995). These findings suggest that the antiatherosclerotic effect of DiNAC may be mediated through an endothelial-dependent mechanism. However, it is to date not possible to establish whether DiNAC and probucol exert the actions described here by the same cellular and/or biochemical processes. It is interesting, for example, that nuclear factor-κB activation is dependent on oxidative processes (D'Angio and Finkelstein, 2000; Schoonbroodt and Piette, 2000), and that such activation may affect both the CS reaction and the rate of atherosclerotic lesion development. The structure-activity relationship for DiNAC and its analogs in the CS test suggests that DiNAC may affect some kind of redox process (Särnstrand et al., 1999). It is thus possible that both probucol and DiNAC may operate through the same pathway(s), but this remains to be elucidated. It is of vital importance that the modes of action of DiNAC (and probucol) are clarified.

In conclusion, the present investigation shows that DiNAC prevents lesion development in WHHL rabbits and may thus represent a new class of drugs that can prevent atherosclerosis-related diseases. The drug is presently investigated further in both animal models of atherosclerotic disease as well as in clinical trials. It is also shown that probucol has an immunomodulatory effect similar to DiNAC as assessed by the CS reaction in mice. We therefore suggest that such an effect of probucol (and certain other antioxidants), rather than antioxidant properties in general, explain or contribute to explain its antiatherosclerotic effect in WHHL rabbits.

References

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