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Effect of Allopurinol on the Ethanol-induced Oxidative Stress : Mechanism of Allopurinol Action

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Abstract

An acute ethanol load(50mmol/kg, i.p.) resulted in an increase in lipid peroxidation and a decrease in the levels of α-tocopherol and ascorbate in rat cerebellum. Pretreatment with allopurinol(146μmol/kg, i.p.) prevented the ethanol-induced increment in lipid peroxidation and decrease in α-tocopherol content. However, the decrease of ascorbate was of greater magnitude when allopurinol was associated with ethanol. These results suggested that allopurinol, besides its action as a radical scavenger and xanthine oxidase inhibitor, might favor the regeneration of α-tocopherol by ascorbate. Therefore, the influence of allopurinol on the monoelectronic exchanges involved in α-tocopherol antioxidant activity was studied using γ-radiolysis in aerated ethanolic solutions. Even though allopurinol did not react by itself with α-hydroxyethyl-peroxyl radicals [H_3C-CH(OH)OO$^{\cdot}$], it enhanced the α-hydroxyethyl-peroxyl radical scavenging properties of α-tocopherol. The regeneration of α-tocopherol from the α-tocopherol radical by ascorbate remained as efficient in the presence of allopurinol as in its absence. The effects of allopurinol on the vitamin E oxidation-reduction mechanisms could be involved in the beneficial effect of allopurinol on the biological cellular damages linked to free radical reactions.

Key words: ethanol, allopurinol, α-hydroxyethyl-peroxyl radical, α-tocopherol

INTRODUCTION

It is generally assumed that an oxidative stress resulting from generation of free radicals in an amount exceeding the capacity of the cellular defense systems plays an important role in the pathogenesis of ethanol-induced liver injury(1). Recent studies have reported that ethanol administration to rats can also cause free radical-mediated oxidative cellular damage in extrahepatic tissues, such as the brain(2-4). Chronic ethanol treatment induced oxidative DNA damage in the hipocampus and cerebellum of rats(2). Dietary administration of ethanol to rats for 2 weeks depressed levels of glutathion and Cu/Zn superoxide dismutase in several brain regions(3). Our previous studies have also shown that an acute ethanol load to rats increased lipid peroxidation in the cerebellum and decreased the concentrations of vitamin E, the major antioxidant against peroxidative degradation of membrane lipid, and vitamin C which regenerates vitamin E from oxidation product(4).

Allopurinol(4-hydroxypyrazol(3,4)-pyrimidine), an inhibitor of xanthine oxidase(XO), has been extensively used for the treatment of hyperuricemia, both of gout and secondary to hematological disorders or antineoplastic therapy(5). Additionally, it has been suggested that the inhibition of XO by allopurinol can reduce the formation of free radicals, and thereby may prevent or ameliorate cellular injuries(6-8). However, some reports have suggested that the beneficial effects of allopurinol during ischemia/reperfusion(9) or whole body γ-irradiation(10) are due to the direct free radical scavenging properties of the drug rather than to its ability to inhibit XO. As a matter of fact allopurinol and its metabolite, oxypurinol, are powerful scavengers of hydroxyl radical($^{\cdot}$OH)(11) and free radicals generated by activated leucocytes (9,12). It has also been demonstrated that allopurinol can facilitate electron transport occurring during the oxidation-reduction mechanisms of cytochrome c(13).

These reports suggest that beneficial effects of allopurinol may be not related only to XO inhibition. It appears that allopurinol can also provide protection against oxidative cellular damage by scavenging free radicals or by other mechanisms.

Therefore, the present study was undertaken first to assess the possible protective effect of allopurinol on the ethanol-induced alterations in lipid peroxidation and contents of vitamin E and vitamin C in cerebellum, a brain region known to be particularly vulnerable to

alcohol intoxication(2). The results obtained from first experiments allowed to hypothesize that allopurinol could act on the oxidation-reduction mechanisms of vitamin E(α-tocopherol) and/or vitamin C(ascorbate). Thus, this possibility was observed in vitro in the second part of the experiments.

It is known(14,15) that α-tocopherol(TH) reacts with peroxyl radicals($ROO^{\cdot}$) according to the following reaction:

$$TH + ROO^{\cdot} \longrightarrow T^{\cdot} + ROOH$$

α-Tocopherol may be regenerated from the tocopheroxyl radical($T^{\cdot}$) by ascorbate(AH^{-})(15,16) according to the following reaction:

$$T^{\cdot} + AH^{-} \longrightarrow TH + A^{\cdot}$$

α-Tocopherol and ascorbate oxidation-reduction radical mechanisms have been studied previously by means of γ-radiolysis in ethanolic solution(17,18). This useful method was used in this study to characterize the possible influence of allopurinol on these mechanisms.

γ-Irradiation of ethanol provides in the nanosecond time scale homogeneous solutions of α-hydroxyethyl radicals($H_3C-{}^{\cdot}CH-OH$) which are represented by $R^{\cdot}$ and of solvated electrons e^{-}_{solve} with known yields:

$G(R^{\cdot}) = 4.8$ molec/100eV
$G(e^{-}_{solv}) = 1.7$ molec/100eV

When γ-irradiation is performed in the presence of air, it provides superoxide anion radicals(O_2^{-}) and the α-hydroxyethyl-peroxyl radicals [$H_3C-CH(OH)OO^{\cdot}$] as model peroxyl radical species $RO^{\cdot}_2$ through the following reactions:

$$R^{\cdot} + O_2 \longrightarrow RO^{\cdot}_2 \qquad k=4.6\times10^{9} mol^{-1}\ l\ s^{-1}$$
$$e^{-}_{solv} + O_2 \longrightarrow O^{-}_2 \qquad k=1.5\times10^{10} mol^{-1}\ l\ s^{-1}$$

MATERIALS AND METHODS

Chemicals

α-Tocopherol, α-tocopherol acetate and ascorbic acid (AH_2) were obtained from Merck(Damstradt, Germany). Allopurinol(All) and 2-thiobarbituric acid were purchased from Sigma Chemical Co.(St. Louis, MO, USA). The absolute ethanol and solvents used for high performance liquid chromatography(HPLC) analysis were obtained from Prolabo(Paris, France).

Animals and treatments

Male Sprague-Dawley rats were obtained from the Laboratory Animal Center of Seoul National University. They were housed in ordinary cages and allowed free access to water and standard diet pellets. The rats were maintained in a temperature(22±2℃), humidity(relative humidity, 55±10%), and light(12 h light, 12 h dark) controlled room. After one week of adaptation period the rats(average weight 180g) were divided into four groups: control, ethanol, allopurinol and ethanol+allopurinol treated group. Ethanol(50mmol/kg b.w.) as a 20%(v/v) solution was administered by intraperitoneal(i.p.) injection to overnight-fasted rats. Pretreatment with allopurinol(146μmol/kg b.w., i.p.) was conducted 16 h and 20 min prior to the ethanol treatment. Control animals were injected with the same volume of saline. The rats were killed by decapitation 4 h after ethanol treatment, and the brain was removed and placed on an iced plate. The cerebellum was rapidly dissected, cleaned of adhering blood, frozen and kept in liquid nitrogen. Cerebellum was used for determination of the lipid peroxidation and the α-tocopherol and ascorbate contents.

Determination of lipid peroxidation

The cerebellum was homogenized(1:40) in phosphate buffer(20 mM, pH 7.4), centrifuged(1000×g, 10 min, 4℃), and incubated for 30 min under air at 37℃ in a shaking water bath. Aliquots of incubated mixture were taken every 5 min to determine the rate of lipid peroxidation, lipid peroxides being quantified by the thiobarbituric acid test(19).

Determination of α-tocopherol

Cerebellar concentration of α-tocopherol was determined by HPLC method(20) with α-tocopherol acetate as internal standard.

Determination of ascorbate

Cerebellar concentration of ascorbate was determined according to Cooper et al.(21).

Determination of protein

Protein was determined according to Lowry et al.(22).

Statistical analysis

All results obtained from *in vivo* studies are given as

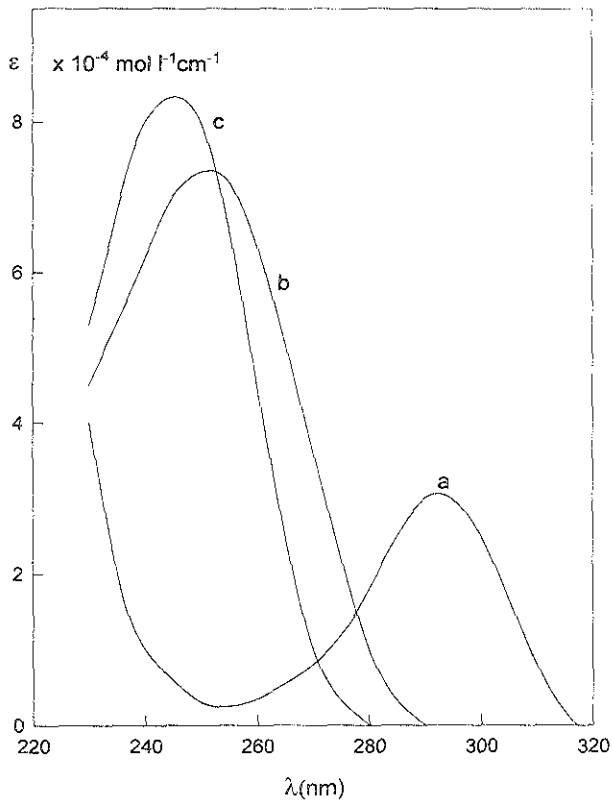

Fig. 1. Absorption spectra: α-tocopherol(spectrum a; λ_{max}=292, ε_{292}=3,150mol^{-1} l cm^{-1}), allopurinol (spectrum b; λ_{max}=250, ε_{250}=7,660mol^{-1} l cm^{-1}) and ascorbate(spectrum c; λ_{max}=245, ε_{245}= 8,500mol^{-1} l cm^{-1}) in ethanol.

mean±S.D. Statistical analysis was performed using a Statview 512^{+} package program(Brainpower Inc., Calabasas, CA, U.S.A.), an analysis of variance. For the differences among the groups, Scheffe F-test was used. A p value less than 0.05 was considered statistically significant.

Analytical methods for γ-radiolysis studies

Aerated ethanol solutions of α-tocopherol, ascorbate and allopurinol were made by vigorous stirring under air. All the titrations were made using a Uvikon 820 spectrophotometer with a 1cm optical pathway: λ_{max}(TH) =292nm; ε_{292}=3.15×10^{3}mol^{-1} l cm^{-1}; λ_{max}(AH_2)=245nm; ε_{292}=8.5×10^{3}mol^{-1} l cm^{-1}(all titrations of ascorbic acid were made after acidification of the medium, H_2SO_4=8×10^{-3}mol l^{-1}) and λ_{max}(All)=250nm; ε_{250}=7.66×10^{3}mol^{-1} l cm^{-1}(Fig. 1). γ-Irradiations were made in a ^{60}Co irradiator. The dosimetry was determined according to Jore et al.(23): radiooxidation of acidic(H_2SO_4, 0.4mol l^{-1}) ferrous sulfate solutions (Mohr salt, 10^{-3}mol l^{-1}) under air atmosphere, taking λ_{max}(Fe^{3+})=304nm, ε_{304}=2204mol^{-1} l cm^{-1} at 25°C and with a yield G(Fe^{3+})=15.6molec/100eV. The doses were provided at a rate of 1.1×10^{18}eVcm^{-3}h^{-1} and used for yield calculations without solvent corrections(23). The glassware used for irradiations was heated at 400°C for 4h after washing to burn out impurities.

RESULTS

Effects of pretreatment with allopurinol

The rate of lipid peroxidation was determind by measuring thiobarbituric acid reactive substances(TBARS) during incubation of tissue homogenates. The rate of TBARS formation was increased significantly 4 h after ethanol administration(Table 1). However, pretreatment with allopurinol prevented this increase.

α-Tocopherol and ascorbate contents of cerebellum decreased significantly 4 h after ethanol administration (Table 2). Whereas allopurinol alone had no effect on these levels, pretreatment with allopurinol prevented the decrease in α-tocopherol but potentiated the decrease in ascorbte induced by ethanol.

Table 1. Effect of allopurinol on the cerebellar lipid peroxidation following an acute ethanol load

Treatments	TBARS(nmol/min/g protein)
Control	72±8 (7)^{a}
Ethanol	94±9 (7)^{b}
Allopurinol	77±10(6)^{a}
Ethanol+Allopurinol	63±9 (9)^{a}

Ethanol(50mmol/kg, i.p.) was injected into rats 4 h before sacrifice. Allopurinol(146μmol/kg, i.p.) was treated 16 h and 20 min prior to the ethanol load. Values are mean±S.D., with the number of animls indicated in parentheses. Statistical significance: values with the different superscripts are significantly different($p<0.05$)

Table 2. Effects of allopurinol on the cerebellar α-tocopherol and ascorbate contents following an acute ethanol load

Treatments	α-Tocopherol (nmol/g tissue)	Ascorbate (μmol/g tissue)
Control	33.4±1.3(8)^{a}	2.09±0.07(21)^{a}
Ethanol	30.9±0.9(10)^{b}	1.93±0.07(16)^{b}
Allopurinol	32.5±1.5(7)^{a}	2.08±0.14(9)^{a}
Ethanol+Allopurinol	33.4±0.7(10)^{a}	1.59±0.16(7)^{c}

Ethanol(50mmol/kg, i.p.) was injected into rats 4 h before sacrifice. Allopurinol(146μmol/kg, i.p.) was treated 16 h and 20 min prior to the ethanol load. Values are mean±S.D., with the number of animls indicated in parentheses. Statistical significance: values with the different superscripts are significantly different($p<0.05$)

Influence of allopurinol on the radical mechanisms involved in the vitamin E antioxidant activity

γ-Radiolysis of aerated ethanolic solutions of allopurinol

In order to observe a possible influence of allopurinol on the oxidation-reduction mechanisms of α-tocopherol and/or ascorbate it was necessary to examine first the reaction of O_2^- and $RO_2^\cdot$ with allopurinol in the absence of α-tocopherol.

When irradiation with doses between 1.2×10^{18} and 4.8×10^{18}eV cm^{-3}, ethanolic soultions of allopurinol ($[All]_o=10^{-4}$ mol^{-1}) in aerobic conditions, no evolution of the absorption spectrum of allopurinol could be observed (results not shown). This indicated that O_2^- and $RO_2^\cdot$ did not react with allopurinol in the considered concentration and dose range.

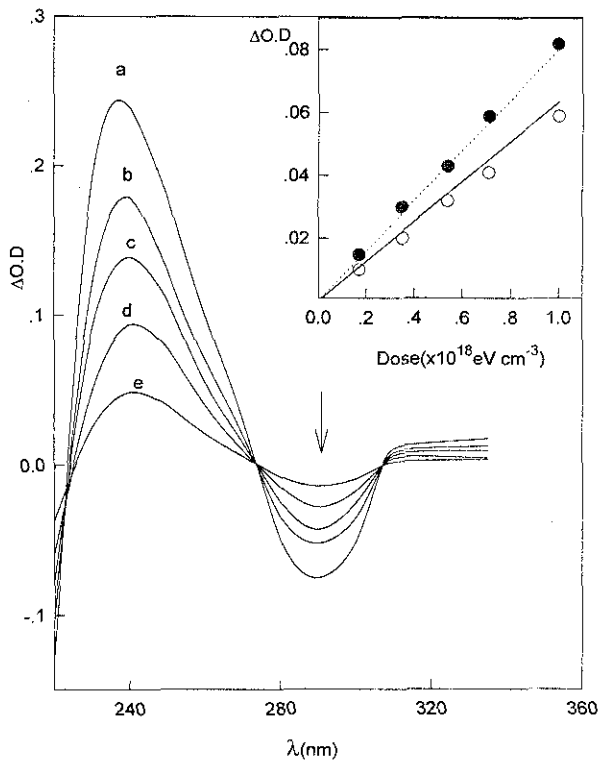

Fig. 2. Differential absorption spectra of TH+All ethanolic solutioins γ-irradiated in aerobic conditions. $[TH]_o=5\times10^{-5}$mol l^{-1}, $[All]_o=10^{-4}$mol l^{-1}. Reference: initial solution. Dose expressed in 10^{18}eVcm^{-3}, (a) 0.17, (b) 0.35, (c) 0.54, (d) 0.71, (e) 1.0. (Dose rate : 1.1×10^{18}eV$cm^{-3}h^{-1}$). Inset: Differential optical density ΔOD_{292} plotted versus dose: -○-: without All, -●-: with All.

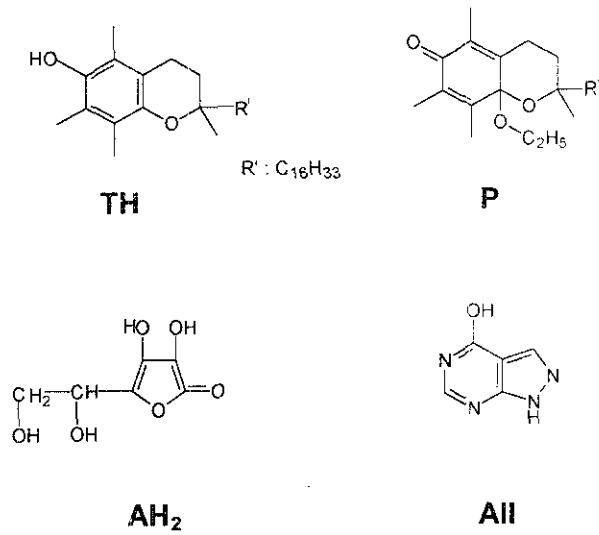

Fig. 3. Molecular structures: α-tocopherol(TH), α-tocopherol oxidation product(P), ascorbic acid (AH_2) and allopurinol(All).

γ-Irradiations of aerated ethanolic solutions of allopurinol and α-tocopherol

γ-Irradiations of mixtures of TH+All were performed in aerated ethanolic solutions. All experiments have been performed with a constant initial allopurinol concentration ($[All]_o=10^{-4}$mol l^{-1})and different α-tocopherol concentrations (ranging from $[TH]_o=2.5\times10^{-5}$ to 5×10^{-4}mol l^{-1}).

Fig. 2 showed as an example the evolution of the differential absorption spectra obtained when irradiation TH+All mixtures($[All]_o=10^{-4}$mol l^{-1} and $[TH]_o=5\times10^{-5}$mol l^{-1}] with doses between 1.8×10^{17} and 1.1×10^{18}eV cm^{-3}. It can be observed that the optical density at λ =292nm decreased according to the dose, while a new absorption maximum appeared at λ =242nm. The presence of isobestic points indicated that α-tocopherol was converted into a single product P which appears to be identical to the α-tocopherol oxidation product previously obtained by radiooxidation of α-tocopherol in aerated ethanolic medium(16)(λ_{max}=242nm, $\varepsilon_{242}=8.5\times10^3$mol^{-1} l cm^{-1}; see structure on Fig. 3). The inset of Fig. 2 indicated that the differential optical density ΔOD_{292} increased linearly as a function of the dose.

As at λ =292nm, only α-tocopherol and its oxidized product P exhibited a significant absorption, the radiolytic yield of α-tocopherol disappearance can therefore be calculated:

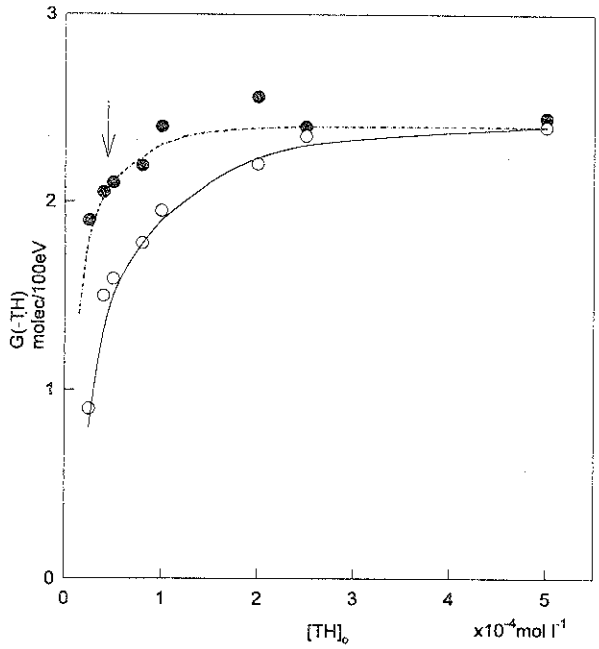

Fig. 4. Dilution curve G(-TH) plotted versus $[TH]_o$. -○- : without All, -●- : with All. The data represent mean values of at least three separate experiments. ↓ : This experimental point was also obtained with $[All]_o=2\times10^{-5}$mol l^{-1}.

$$G(-TH)=\frac{\frac{\Delta OD_{292}}{\Delta \varepsilon_{292}}\times N\times 0.1}{dose}$$

with $\Delta \varepsilon_{292}=\varepsilon_{292}(TH)-\varepsilon_{292}(P)$

$N=6.02\times10^{23}$

The dose was expressed in eVcm^{-3}; 0.1 corresponds to a unit correcting factor. G(-TH) in this case was equal to 2.1molec/100eV. It could also be seen(inset of Fig. 3) that this value was above the one obtained when α-tocopherol was irradiated in the absence of allopurinol : G(-TH)=1.6molec/100eV.

Analogous experiments were performed for other (TH) concentrations chosen in the above range. The experimental results showed that G(-TH) values were higher than the yields obtained when α-tocopherol was irradiated in the absence of allopurinol as can be seen on Fig. 4. Furthermore the plateau of this dilution curve was the same, but it was reached for concentrations of α-tocopherol above 10^{-4}mol l^{-1} in the presence of allopurinol (above 2×10^{-4}mol l^{-1} in its absence).

Irradiations of aerated ethanolic solutions of allopurinol and ascorbate

These experiments have been performed in the same way as reported when studying the radiooxidation of ascorbate(represented here by AH^-) in aerated ethanolic solution. This radiooxidation led to the formation of dehydroascorbic acid (A)(18).

As an example, the results obtained after irradiation of a AH^- + All mixture in an aerated ethanolic solution ($[AH^-]_o=10^{-4}$mol l^{-1}, $[All]_o=10^{-4}$ mol l^{-1}) were presented on Fig. 5. It can be observed that the optical density at 245nm decreased according to the dose. The inset of this figure indicates that the optical density OD_{245} decrease linearly as a function of the dose in the same way in the presence of allopurinol and in its absence. The yield $G(-AH^-)$ was calculated and still equal to 3.2molec/100eV=1/2($GRO^{\cdot}_2+GO_2^-$). These results suggested that allopurinol did not influence the yield of ascorbate oxidation by $RO^{\cdot}_2$ and O_2^- free radicals in the considered concentration and dose ranges.

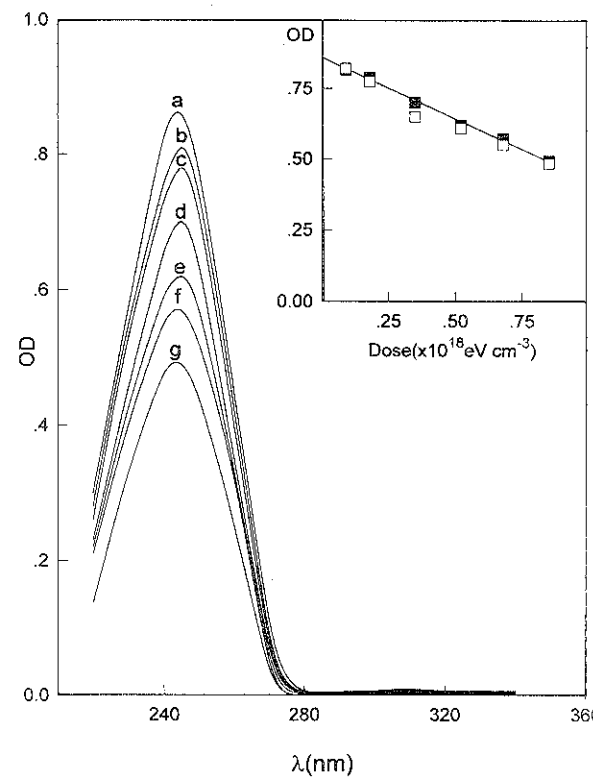

Fig. 5. Absorption spectra of All+AH^- aerated ethanolic solutions.
$[All]_o=10^{-4}$mol l^{-1}, $[AH^-]_o=10^{-4}$mol l^{-1}. Reference : solution containing only All. Titrations of ascorbate were made after acidification of the medium with H_2SO_4(8×10^{-3}mol l^{-1}). Dose(10^{18}eVcm^{-3}): (a) initial solution (b) 0.09, (c) 0.18, (d) 0.35, (e) 0.52, (f) 0.68, (g) 0.85. Inset : Optical density OD_{245} plotted versus dose:
-■-: (AH^- + All), -□-: AH

Irradiations of aerated ethanolic solutions of allopurinol, α-tocopherol and ascorbate

γ-Irradiations of All+TH+AH^- mixtures have been performed in aerobic conditions with: $[All]_o=1\times10^{-4}$mol l^{-1}, $[AH^-]_o=5\times10^{-5}$mol l^{-1} and $[TH]_o=5\times10^{-5}$mol l^{-1}. The measurement of the differential optical density at 292nm allowed to follow the evolution of α-tocopherol.

It can be seen on Fig. 6 that α-tocopherol disappeared according to the absorbed dose between 0.18 and 1.10^{18}eVcm^{-3}. The inset of this figure indicated that the differential optical density ΔOD_{292} increased linearly as a function of the dose, and allowed the calculation of the yield G(-TH). G(-TH) was equal to 1.0molec/100eV either in the presence or the absence of All. This value was identical to the one obtained when α-tocopherol and ascorbate were irradiated in the same conditions of dose and concentration in the absence of allopurinol(17,18).

DISCUSSION

It is generally assumed that allopurinol prevents free radical-mediated damage through its inhibitory effect on XO. Since the activity of xanthine oxidase is very low in the central nervous system(8,24), it appears unlikely that the inhibition of XO represents the main mechanism responsible for the protective effect of allopurinol on the oxidative stress caused by an acute ethanol.

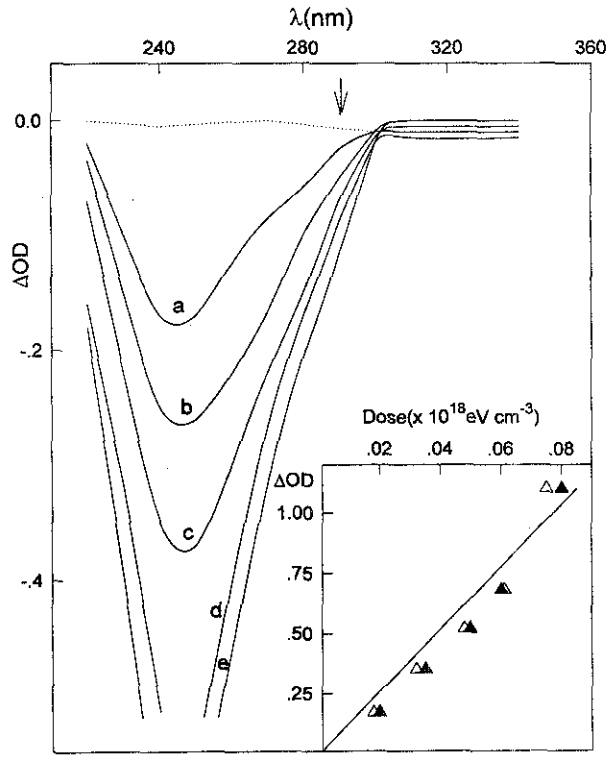

Fig. 6. Differential absorption spectra of All+TH+AH^- aerated ethanolic solutions.
$[All]_o=10^{-4}$mol l^{-1}, $[TH]_o=5\times10^{-5}$mol l^{-1}, $[AH^-]_o=5\times10^{-5}$mol l^{-1}. Reference: initial solution. Dose (10^{18} eVcm^{-3}): (a) 0.17, (b) 0.35, (c) 0.52, (d) 0.68, (e) 1.1. Inset: Differential optical density ΔOD_{292} plotted versus dose:
-▲-: All+TH+AH^-, -△-: TH+AH^-

1) Interactions between allopurinol and α-tocopherol

Upon irradiation with ^{60}Co γ-rays in an aerated ethanolic medium, α-tocopherol was oxidized into its product P with a yield G(-TH) which increased with $[TH]_o$ concentration and reached a constant value equal to $G(RO_2^\cdot)/2$=2.4molec/100eV(Fig. 4). The following mechanisms have been established to fit the experimental data(17):

(1) $RO_2^\cdot + TH \longrightarrow T^\cdot + ROOH$, $k_1=9.1\times10^4$mol^{-1} l s^{-1}

(2) $RO_2^\cdot + T^\cdot \xrightarrow{H^+} T^+ + ROOH$, $k_2=2.5\times10^6$mol^{-1} l s^{-1}

(3) $T^\cdot + T^\cdot \xrightarrow{H^+} TH + T^+$ $k_3=1\times10^4$mol^{-1} l s^{-1}

(4) $RO_2^\cdot + RO_2^\cdot \longrightarrow$ *non radical products* $k_4=3\times10^8$mol^{-1} l s^{-1}

(5) $T^+ + C_2H_5OH \longrightarrow P + H^+$ $(P=TOC_2H_5)$

Reaction (4) competes with reaction (1) and (2) only when $[TH]_o$ is below 2×10^{-4}mol l^{-1} and lowers G(-TH).

In the presence of allopurinol, the oxidation of α-tocopherol by peroxyl radicals $RO_2^\cdot$ generated the same product P as that observed previously in the absence of allopurinol. The value G(-TH) obtained when the plateau of the dilution curve was reached stays equal to $G(RO_2^\cdot)/2$=2.4molec/100eV. These results lead to conclusion that two $RO_2^\cdot$ radicals were used for the oxidation of one α-tocopherol molecule affording first the T^+ species which reacts with the solvent to provide the final product P.

However the fact that the constant value G(-TH) was reached more rapidly in the presence of allopurinol, suggested that reaction (4) competed with reactions different from reactions (1) and (2). As allopurinol did not react directly with $RO_2^\cdot$, such an effect could be linked to the possible interaction of allopurinol with either α-tocopherol or its oxidized radical.

Two hypotheses may therefore be proposed:

Hypothesis 1:

Allopurinol is capable of providing a complex with α-tocopherol according to:

(6) TH + All ⟶ TH-All

The monoelectronic oxidation of TH-All would provide a $T^{\cdot}$ radical according to:

(7) $(TH\text{-}All) + RO_2^{\cdot} \longrightarrow T^{\cdot} + All + ROOH$

$T^{\cdot}$ would then lead to the final product P through reactions (2), (3) and (5). In this case $G(-TH)_{max}=G(P)_{max}$ is equal to $G(RO_2^{\cdot})/2$. For concentrations of $[TH]_o$ below 10^{-4}mol l^{-1}, the observed competition concerns reactions (7) and (4) in favor of reaction (7).

Hypothesis 2:

Allopurinol is able to react with the α-tocopherol oxidized radical($T^{\cdot}$) according to:

(8) $T^{\cdot} + All \longrightarrow (T\text{-}All)^{\cdot}$

Then $(T\text{-}All)^{\cdot}$ would evolve preferentially by a second oxidation by $RO_2^{\cdot}$ according to:

(9) $(T\text{-}All)^{\cdot} + RO_2^{\cdot} \xrightarrow{H^+} T^+ + All + ROOH$

Competition between reactions (9) and (4) in favor of (9) would increase the yield G(-TH) compared to the values obtained in the absence of allopurinol with the competition between reactions (1), (2) and (4) for $[TH]_o < 2\times10^{-4}$ mol l^{-1}.

Any other mechanism involving a consumption of $T^{\cdot}$ by All, such as (10):

(10) $T^{\cdot} + All \longrightarrow All^{\cdot} + T^-$

is to be excluded due to the fact that $G(-TH)_{max}=2.4$ molec/100eV.

In fact reactions (1), (10) and (5) would lead to $G(-TH)_{max}=4.8$molec/100eV.

It can therefore be concluded that allopurinol enhanced the scavenging properties of α-tocopherol on the $RO_2^{\cdot}$ radicals in our experimental conditions of doses and concentrations.

2) Interactions between allopurinol, α-tocopherol and ascorbate

It has been shown previously(18) that the results of irradiations of TH+AH mixtures in aerated ethanolic solution depended only on the ratio $[TH]_o/[AH^-]_o$. This study pointed out the reaction (11) of TH regeneration by AH^- from its oxidized radical $T^{\cdot}$:

(11) $T^{\cdot} + AH^- \longrightarrow TH + A^-$
$k_{11}=6\times10^5$ mol^{-1} l s^{-1}

as well as an oxidation of $T^{\cdot}$ by dehydroascorbic acid(A) arising from reaction (12) according to reaction (13):

(12) $A^- + A^- \longrightarrow A + AH^-$

(13) $T^{\cdot} + A \longrightarrow T^+ + A^-$
$k_{13}=1.2\times10^7$ mol^{-1} l s^{-1}

In order to suggest an interpretation of the results obtained when irradiating All+TH+AH^- mixtures, 2 facts are to be considered:

1) For a ratio $[TH]_o/[AH^-]_o$ equal to 1, the yield G(-TH) stays equal to 1molec/100eV in the absence of All or in its presence ($[All]_o=10^{-4}$ mol l^{-1}).

2) In the absence of AH^-, G(-TH) in the presence of All is above the G(-TH) value obtained without All(for $[TH]_o < 2\times10^{-4}$ mol l^{-1}). These results indicate that, in the presence of All, the regeneration of TH by AH^- remains as efficient as in the absence of All. It seems therefore reasonable to suggest that:

- According to hypothesis 1(presented above), the regeneration of TH results still from the competition between reactions (11) and (13) which is the same with or without All.

- According to hypothesis 2, the regeneration of TH might involve either a reaction as (14):

(14) $(T\text{-}All)^{\cdot} + AH^- \longrightarrow A^- + TH + All$

which would compete with reaction (15) :

(15) $(T\text{-}All)^{\cdot} + A \longrightarrow A^- + T^+ + All$

or a competition between reactions (8), (11) and (13) favorable towards reactions (11) and (13) which would almost suppress any interaction of allopurinol in this radical mechanisms.

This study indicated that allopurinol enhanced the scavenging properties of α-tocopherol on α-hydroxyethylperoxyl radicals, and that the regeneration of TH from $T^{\cdot}$ by AH^- stayed as efficient as in the absence of allopurinol for the considered irradiation dose and concentration ranges.

The relevance of the reported results obtained *in vitro* to biological systems is uncertain. However allopurinol administration prevented the increase in cerebellar lipid peroxidation induced in rats by an acute ethanol load. This effect could be linked to the enhancement of peroxyl radical scavenging activity of vitamin E in the presence of allopurinol. In addition, allopurinol prevented the decrease in the vitamin E cerebellar content, whereas it enhanced the decrease in vitamin C level. This could be related to the regeneration of vitamin E at the expenses of vitamin C level.