RESVERATROL, AN ARYL HYDROCARBON RECEPTOR ANTAGONIST, DOES NOT PREVENT TOXICITY FROM 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) IN RATS *IN VIVO*

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Introduction

The AH receptor (AHR) plays an essential role in dioxin toxicity as demonstrated by the great resistance to dioxin toxicity that exists in mice in which the AHR has been genetically knocked out. From the time the AHR first was discovered there has been a search for a chemical that could block AHR function by acting as a "pure" antagonist, both to provide a tool for probing the biological and toxicological roles of the receptor and as a possible chemopreventive agent to reduce the toxicity of dioxins. Compounds that are weak agonists for the AHR can diminish biochemical and toxic responses to TCDD[1,2]. However, most compounds that possess antagonistic properties also exert some weak agonistic effects on AHR-mediated pathways[1-4]. Recently resveratrol, a polyphenolic compound found in grapes, was shown to antagonize biochemical events mediated through the AHR such as CYP1A1 induction without appearing to exert any agonistic effect[5-7]. Thus we tested resveratrol *in vivo* to determine if this AHR-antagonist could reduce or prevent acute toxic responses to TCDD.

Materials and Methods

Male Long-Evans rats (5 weeks old) were given resveratrol (50 mg/kg) by gastric intubation in a DMSO/corn oil vehicle. Four hours later they were given TCDD intragastrically (in corn oil) at a dose that is sublethal in this strain (5 μg/kg) or at a dose that is lethal (50 μg/kg). Resveratrol (or vehicle) was administered once each day for 3 further days before the rats were killed on Day 4. Body weight and food intake were measured daily; thymus and liver were weighed on Day 4. Liver microsomes were prepared for measurement of ethyoxyresorufin O-deethylase activity as an index to induction of CYP1A1.

Results and Discussion

TCDD treatment caused the expected decreases in body weight and thymus weight as well as a more than 30-fold increase in EROD activity [TABLE 1]. TCDD at 50 μg/kg also caused a substantial reduction in food intake on Day 3 and Day 4 [data not shown]. Resveratrol on its own did not affect any of the endpoints that we measured nor did resveratrol have any significant effect on the TCDD-induced toxic responses (food intake, body weight, thymus weight, liver weight) or biochemical response (EROD activity) [TABLE 1]. We also tested the effect of resveratrol in a second rat strain, the Han/Wistar(*Kuopio*) strain that is highly resistant to the lethal effects of TCDD but a strain in which CYP1A1 induction remains normal[8]. In Han/Wistar(*Kuopio*) rats given 0.5 μg/kg TCDD the EROD activity in liver was induced 20-fold; however, as was true with Long-Evans rats, resveratrol (50 mg/kg) did not prevent induction of EROD [data not shown].

TABLE 1

Treatment	Body Weight Day 4 [g]	Thymus Weight [mg]	Liver Weight [g]	EROD Activity
Vehicle	120 ± 8.3	208 ± 66	5.6 ± 0.53	35 ± 13.7
Resveratrol	116 ± 10	208 ± 38	5.3 ± 0.66	36 ± 17.6
TCDD 5 μg	111 ± 7.6	142 ± 26	6.7 ± 0.65	1166 ± 277
TCDD 5 μg plus Resveratrol	108 ± 7.0	140 ± 33	6.4 ± 0.81	1043 ± 465
TCDD 50 μg	98 ± 5.6	107 ± 38	6.0 ± 0.40	559 ± 209
TCDD 50 μg plus Resveratrol	99 ± 11	90 ± 29	5.8 ± 0.76	506 ± 208

Values in the table are mean ± SD; n = 6 for all observations.

EROD activity is expressed as pmol/min/mg microsomal protein.

To confirm that resveratrol was capable of inhibiting binding of TCDD to the AHR we performed a binding experiment in which we tested the ability of resveratrol to compete with [^{3}H]TCDD for binding to AH receptor in a cytosolic preparation *in vitro*. Resveratrol completely inhibited [^{3}H]TCDD binding at a resveratrol concentration of 100 micromolar; the IC50 for inhibition was approximately 8 micromolar in competition against 5 nM [^{3}H]TCDD [data not shown].

The lack of an antagonistic effect of resveratrol *in vivo* was surprising in light of resveratrol's well-documented ability to inhibit AHR-mediated responses such as induction of CYP1A1[5-7]. An obvious question regarding the lack of inhibition of responses to TCDD in our study is whether the administered dose of resveratrol (50 mg/kg i.g.) was effectively absorbed and distributed to the target tissues. The dose of resveratrol that we used was substantially above the level employed in previous *in vivo* studies[7,9] of various biochemical responses, yet resveratrol treatment still had no measurable impact upon responses to TCDD.

We do not yet know the level of resveratrol that was achieved and maintained in blood or tissues following the 50 mg/kg dose; however, previous pharmacokinetic studies after oral dosing in rats[10] indicate that the 50 mg/kg dose of resveratrol would be expected to lead to high levels of resveratrol in liver and other tissues.

Ciolino and coworkers[5,6] were first to report that resveratrol could inhibit TCDD-induced transcription of the *CYP1A1* gene and that resveratrol inhibits induction of CYP1A1 by BP or DMBA in human HepG2 hepatoma cells in culture. No studies were performed *in vivo* to test the ability of resveratrol to block biochemical or toxic responses to TCDD. Casper *et al.*[7] found that resveratrol was an effective inhibitor of TCDD-induced transactivation of gene expression *in vitro* and CYP1A1 induction in cell culture; they also reported that resveratrol totally suppressed CYP1A1 induction in liver, lung and kidney of rats treated *in vivo* with a mixture of benzo[*a*]pyrene (BP) and 7,12-dimethylbenz[*a*]anthracene (DMBA). However, the effect of resveratrol on CYP1A1 induction by TCDD was not tested in these experiments.

It could be argued that the results of our experiments with resveratrol indicate that the AHR does not mediate toxicity of TCDD in this rat model. There is, however, ample evidence from genetic studies in these rats[11] to demonstrate that the AHR is a key component in the dioxin toxicity mechanism. Furthermore it is well-established from hundreds of studies that the AHR mediates CYP1A1 induction as well as most other responses to dioxin-like chemicals[8,12,13].

It has been proposed[7] that resveratrol might be useful clinically to prevent adverse effects from dioxin-like chemicals. Certainly resveratrol is highly effective at blocking AHR-mediated CYP1A1 induction in cell culture as well as certain other AHR-mediated responses [5-7]. There is no question that resveratrol is able to inhibit binding of TCDD to the AHR as shown in our experiments and those of Casper *et al.*[7]. The results of our study are disappointing in the sense that resveratrol was unable to prevent biochemical or toxic

responses to TCDD in this first test versus TCDD in an *in vivo* model. However, since resveratrol is a highly effective AHR-antagonist *in vitro* and in cell culture and since resveratrol is a natural product which appears to have little risk ofoxicity itself, further *in vivo* studies are warranted to attempt to determine if resveratrol might be a useful chemopreventive agent under a suitable treatment regimen.

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