Neuroprotective agents for clinical trials in Parkinson's disease: A systematic assessment

To the Editor: We read with interest the Special Article by Ravina et al. proposing neuroprotective candidate drugs for clinical trials in Parkinson's disease (PD).[1] Minocycline, which has already been launched for phase II/III trials in PD, Huntington's disease (HD), and atypical parkinsonism, is one of the 21 selected drugs that passed a set of predefined evaluation criteria: primary mechanism(s), consistency of preclinical data, blood–brain barrier penetration, safety/tolerability ratio, and relevant animal model efficacy. The pharmacokinetics and mechanisms of anti-inflammatory/antiapoptotic actions of minocycline are well known.[1] There is some evidence that minocycline might confer neuroprotection in rodent models of PD-like neurodegeneration and transgenic model of HD, using various doses and routes of administration.[1,2]

In contrast with those latter, our own experiments fail to show beneficial effects of minocycline in three different animal models (unpublished data). Using a progressive 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) nonhuman primate model that replicates the progressive nature of PD,[3] we observed that minocycline (200 mg twice daily, 12 hours apart, minocycline generic) treatment had a deleterious effect. Indeed, while MPTP-placebo-treated animals displayed mild parkinsonism at day 15 (mean motor score = 5 ± 0.6), the minocycline/MPTP-treated tended to be more affected (11 ± 1.2, $p = 0.057$, Mann-Whitney), suggesting that minocycline/MPTP-treated animals developed symptoms more rapidly and severely.

Striatal sections from both groups were processed at day 15 for dopamine transporter binding,[2] compared to control animals. The minocycline/MPTP-placebo-treated animals showed a greater loss of striatal dopaminergic nerve endings than MPTP-treated animals (e.g., in dorsal caudate at the rostral level, −76% and −54.5% in comparison with controls; $p < 0.0001$, analysis of variance). We also investigated the effect of minocycline (minocycline HCl, Sigma, 45 mg/kg twice daily in saline, IP) in the systemic subacute 3-nitropropionic acid (3-NP) model of HD in C57B1/6 mice (n = 8: 3-NP + minocycline, n = 8: 3-NP + saline, n = 8: saline + saline controls).[4] The minocycline-treated group was significantly more behaviorally impaired, from day 5 onwards (at the end of the 3-NP intoxication period, total dose of 3-NP = 360 mg) until being killed at day 13 (3-NP + minocycline = 2.6 ± 0.1 vs 3-NP + saline = 1.0 ± 0.1, $p < 0.0001$, vs controls 0.2 ± 0.05, $p < 0.0001$, Mann-Whitney).

During the first week after 3-NP intoxication the behavioral performance of minocycline-treated mice as measured by rotarod, pole test, traversing a beam tasks, and general activity parameters was also significantly decreased ($p < 0.05$ compared to 3-NP alone and controls, unpaired *t*-test). Not surprisingly, striatal cell loss was more severe in the minocycline-treated mice. Interactions between minocycline and 3-NP were excluded by the study of brain SDH (mitochondrial complex II) inhibition. In the third experiment we studied the effect of minocycline (15 mg/kg twice daily) in the "double toxin-double lesion" rat model of striatonigral degeneration (SND/MSA-P), using sequential stereotactic injection of 6-hydroxydopamine (6OHDA) in the medial forebrain bundle (MFB) and quinolinic acid (QA) in the striatum.[5] Wistar rats randomly selected in minocycline treated (n = 15) and untreated groups (n = 15) received stereotaxic QA injection (90 nmol) into the left striatum and 3 days later 6OHDA (8 μg) in the left MFB. Minocycline-treated animals were injected IP before the surgeries and every day for 3 weeks. Minocycline-treated and untreated animals were not different whatever the considered parameter; i.e., locomotor activity and striatal cell loss (DARPP-32).

Although minocycline significantly suppressed astroglial (GFAP) and microglial (Ox6) activation, only a marginal neuroprotective effect at a single level of the ipsilateral substantia nigra (mean neuronal count 43.7 ± 10.7 in the minocycline group vs 30.7 ± 18 in the saline group, $p < 0.05$, unpaired *t*-test) has been observed. A stereologic analysis of the whole substantia nigra failed to elicit a positive effect. Taking into account these results in three different neurodegenerative models, we disagree with Ravina et al.[1] considering that there is consistent animal experimental basis supporting neuroprotective effects of minocycline in PD, HD, and atypical parkinsonism. We believe that additional experimental work should be considered to establish the scope and consistency of minocycline neuroprotective effects in neurodegenerative basal ganglia disorders before embarking on further clinical trials.

E. Diguet, C.E. Gross, PhD, E. Bezard, PhD,
F. Tison, MD, PhD, *Bordeaux, France;* N. Stefanova, MD, PhD,
G.K. Wenning, MD, PhD, *Innsbruck, Austria*

Reply from the Authors: We appreciate the input of Diguet et al. and strongly encourage the submission of all relevant information on potential drugs for PD to the Committee to Identify Neuroprotective Agents (CINAPS). However, published data clearly support pilot studies of minocycline in PD at this time.

The comments from Diguet et al. raise several issues about the way drugs are selected for testing in clinical trials. The CINAPS process emphasized the need for consistent preclinical results.[1] This is a higher level of evidence than is often used in drug selection and drugs are often selected for trials on the basis of a single positive preclinical study. Minocycline has been shown to have neuroprotective effects in published studies of MPTP[6,7] and six OHDA[8] treated rodents, transgenic rodent models of both amyotrophic lateral sclerosis (ALS)[9-12] and HD,[13,14] and in excitotoxicity models.[15,16] The mechanisms of inhibition of glial activation and apoptosis are relevant in PD and minocycline achieves concentrations in the CNS necessary for neuroprotection.[1,2,7,17] These data clearly support the use of minocycline in PD.

In the interest of objectivity, the CINAPS process included only data published in peer-reviewed journals for nonproprietary compounds. We strongly encourage the publication of negative studies, but Diguet et al.'s letter provides an incomplete account of their experiments and unpublished studies such as these are difficult to evaluate fully. The details of the experimental design as well as the results are not fully disclosed. It is difficult then to speculate as to why Diguet et al. obtained results opposite those in the published literature nor do the authors offer an explanation.

The foremost responsibility of any clinical researcher is patient safety and thus we take seriously any possibility that a drug could worsen PD. Minocycline has been used clinically for decades and is currently being studied in ALS and HD without evidence that is hastens neurodegeneration.[18] The National Institute of Neurological Disorders and Stroke (NINDS)–sponsored neuroprotection trials are designed specifically to evaluate safety and determine if minocycline and other agents warrant further study for efficacy.

Diguet et al. suggest that further testing of minocycline in clinical trials should not proceed. But uncertainty and even contradictory evidence are a part of clinical trials and clinical equipoise. Clinical equipoise, or uncertainty about the benefits of a drug in the research community as a whole, allows for the ethical conduct of clinical trials. Far from prohibiting further study, clinical equipoise acknowledges that new information and disagreement are a part of clinical trials. The preponderance of data strongly favors minocycline; this view is shared by the NINDS neuroprotection study steering committee (personal communication). The ultimate value of minocycline can only be determined in human studies. Delaying human study for further testing in preclinical models with uncertain predictive validity is unlikely to decisively determine the role of minocycline in PD.

B. Ravina, MD, S. Fagan, PharmD, R. Hart, MD,
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T. Dawson, MD, PhD, J. Marler, MD, *Rockville, MD*

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Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury

To the Editor: We read with interest the article by Franz et al.[1] on Aβ-42 and tau in CSF after severe traumatic brain injury (TBI). The authors report decreased CSF Aβ-42 in TBI cases compared with patients with dementia and controls. Low CSF Aβ-42 also correlated with poor outcome after TBI. The authors conclude that their findings indicate a possible pathophysiologic role of Aβ in TBI.

Of the 29 CSF samples in their TBI group, 14 were ventricular CSF (V-CSF) samples obtained through a intraventricular catheter placed for intracranial pressure, whereas 15 were lumbar CSF (L-CSF) samples collected by lumbar puncture at different time points (up to 284 days) after trauma. In both control groups, L-CSF was analyzed. In their statistical comparisons, all TBI cases were treated as one group, regardless of whether V-CSF or L-CSF was analyzed.

It is well known that the concentration of different compounds varies markedly between different CSF spaces. For example, the levels of the monoamine metabolites HVA and 5-HIAA are four to five times higher in V-CSF than in L-CSF,[2] and the level of certain peptides is more than 50% lower in V-CSF than in L-CSF.[3] This makes it hazardous to compare groups without presenting V-CSF and L-CSF data separately.

In patients with Alzheimer disease undergoing a treatment trial with intracerebroventricular GM1 ganglioside,[4] we obtained L-CSF and V-CSF samples during the same day. We analyzed Aβ-42 using the same ELISA method as that used by Franz and coworkers. The level of Aβ-42 was markedly lower ($p < 0.001$) in V-CSF (mean + SEM 121 + 54 pg/mL) than in L-CSF (560 + 61 pg/mL). Individual values are shown in the figure.

Therefore, there is an obvious risk that the reduction of Aβ-42 in TBI cases found by Franz et al.[1] may be caused by the physiologically lower Aβ-42 levels in V-CSF in their mixed set of V-CSF and L-CSF samples in their TBI group. As the authors point out, their findings stand in contrast to the study by Raby et al.,[5] in which increased CSF Aβ-42 was found after TBI. It would be interesting if Franz et al. would reply showing the data as in their figure 1, but with the TBI group separated into cases with V-CSF and L-CSF.

It would also be interesting if Franz et al. would specify why V-CSF samples were not taken in all their TBI cases. If V-CSF (in which Aβ-42 is physiologically lower) was only taken in very severe TBI cases in need of an intraventricular catheter for intracranial pressure monitoring, this also may explain the correlation between low Aβ-42 and poor clinical outcome.

Kaj Blennow, MD, PhD, Bengt Nellgård, MD, PhD, *MöIndal, Sweden*

Reply from the Authors: Drs. Blennow and Nellgård make an important point stressing the fact that there is a ventriculolumbar protein gradient in CSF with usually two- to three-fold higher protein levels in lumbar CSF. The gradient for Aβ-42 quoted by Drs. Blennow and Nellgård is unusually high (more than fourfold) and might be attributable to a disturbance of CSF circulation in patients with NPH.

In our study[1] there was a significant difference of Aβ-42 levels between V-CSF (mean 155 pg/mL, SD 53.4 pg/mL) and L-CSF (mean 263 pg/mL, SD 105.6 pg/mL), which is, however, due to poorer outcome in the V-CSF group (mean Glasgow Outcome Scale GOS [GOS] 2.3) than in the L-CSF group (mean GOS 3.1; $p = 0.02$). Relating the Aβ-42 levels to the QAlb (Aβ-42/QAlb) in order to correct for the CSF protein gradient there was no significant difference ($p = 0.46$) between the V-CSF (18.5) and the L-CSF group (23.9).

Applying a slightly higher cutoff value of 240 pg/mL, the sensitivity of Aβ-42 for poor outcome (GOS 1–3) was 92% at a specificity of 100% in the V-CSF group, and the sensitivity was 87% at a specificity of 83% in the L-CSF group. The overall sensitivity using this cutoff value was 91% at a specificity of 86%, suggesting that there was no difference between V-CSF and L-CSF groups.

One should be very cautious with these figures because statistical power of the subgroup analysis is much too low to give meaningful results, which was why we did not show them in the original article.

These results suggest that there is no remarkable ventriculo-

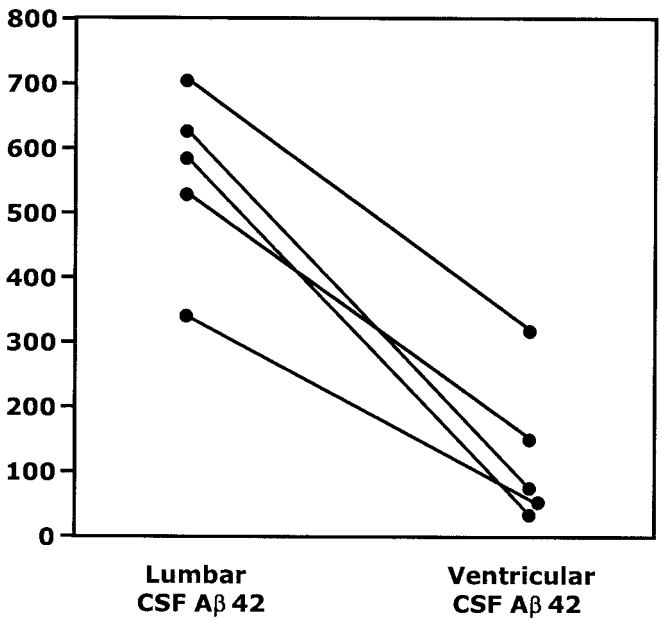

Figure. CSF Aβ-42 in paired lumbar and ventricular CSF samples from five patents with Alzheimer's disease.

lumbar gradient for Aβ-42 in TBI, and lower Aβ-42 levels in the V-CSF subgroup are related to poorer outcome in these patients. To our knowledge, no data have been published concerning CSF circulation after acute TBI. Previous studies found different CSF compounds markedly varying between different CSF compartments in patients with normal pressure hydrocephalus.[2,3] Hydrocephalus is caused by CSF circulation failure. The incidence for post-traumatic hydrocephalus ranges from 0.7% to 29%,[6] and was rarely observed in our patients with TBI because only three of them had enlarged ventricles on CT scans.

G. Franz, MD, R. Beer, MD, A. Kampfl, MD, K. Engelhardt, MD, E. Schmutzhard, MD, H. Ulmer, PhD, F. Deisenhammer, MD, *Innsbruck, Austria*

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Influence of Alzheimer pathology on clinical diagnostic accuracy in dementia with Lewy bodies

To the Editor: In a recent comparative clinicopathologic study of 98 autopsy-proven cases of dementia with Lewy bodies (DLB), Merdes et al.[1] observed that DLB patients with low Braak stages (0–2) had a higher frequency of visual hallucinations and extrapyramidal signs (EPS) but a nonsignificantly higher degree of EPS than those with high neuritic Braak stages (3–6). The clinical diagnostic accuracy for DLB was relatively low (48%), but higher for subjects with lower (75%) than those with higher (38%) Braak stages, suggesting that the degree of concomitant Alzheimer's disease (AD) tangle pathology has an important influence on both clinical features and clinical diagnostic accuracy of DLB.

These data can, at least in part, be confirmed by the results of a personal consecutive series of 96 cases of autopsy-proven DLB. Average age at onset was 67 (SD 12.7) years, and median survival from symptom onset was 5.0 years, 95% CL of median 4.6–5.4, mean 6.7 years.[2] The cohort included 60 cases with low Braak stages (mean 3.5 [range 1.5 to 4]), 24 men, 36 women, with a mean age of 76.9 (range 44 to 90) years, 72% limbic or transitional, and 28% cortical forms; and 36 cases with high Braak stages (mean 3.9 [range 4 to 5]), 15 men, 21 women, mean age 78.7 (range 66 to 91) years.

Sensitivity, specificity, and positive predictive value (PPV) of the McKeith criteria for probable DLB[3] were assessed retrospectively for the total cohort at first neurologic visit (19, SD 19, months) and the last visit (66, SD 55, months after symptom onset). At first visit, sensitivity was 0.22, specificity was 0.97, and PPV was 0.71, but at last visit, sensitivity (0.60) increased, whereas both specificity (0.85) and PPV (0.60) decreased.[4] The presenting clinical symptoms of both limbic and neocortical types of DLB with low Braak stages were EPS alone (36%); psychiatric features, such as visual hallucinations, delusions, and depression, were less frequent at symptom onset. Patients with initial EPS were younger than those without (mean 63, SD 14.6 vs 69, SD 10.6) years.

In contrast, increased age at symptom onset was observed in patients presenting with fluctuating cognition (mean age 75, SD 6.5 vs 66, SD 12.9 years; $p = 0.001$). By contrast, the majority of DLB patients with high Braak stages clinically presented with initial dementia often associated with fluctuating cognition, with or without later development of EPS. Dementia, fluctuating cognition, and hallucinations at symptom onset and shorter latencies to dementia onset strongly predicted shorter survival than initial EPS and longer delay of development of dementia (mean 5.6 vs. 3.3 years, $p < 0.001$).[5]

For all visits, the McKeith criteria better distinguished DLB from PD (possible DLB at first visit 0.72, at last visit 0.86; probable DLB at first visit 1.00, at last visit 0.94) than from AD (possible DLB at first visit 0.67, at last visit 0.45; probable DLB at first visit 0.71, at last visit 0.68).[4] The clinical accuracy for DLB cases with low Braak stages, using the McKeith criteria for probable DLB, similar to the findings by Merdes et al.,[1] was higher (70%) than for patients with severe neuritic AD pathology (high Braak stages) that detected fewer DLB patients (22%) but also included a significant number of subjects with false positive diagnosis (29%).[4]

Both studies, although using somewhat different degrees of Braak staging for the assessment of concomitant neuritic AD pathology and showing differences in the predominant presenting clinical symptoms between DLB cases with mild and severe AD lesions, emphasize the important influence of concomitant neuritic AD pathology on the clinical features, natural history, and the clinical diagnostic accuracy of DLB. However, the pathogenic relationship between Lewy body and Alzheimer pathologies and their relative impact on the clinical course of DLB cases with different degrees of concomitant Alzheimer pathology remain to be elucidated.

Kurt A. Jellinger, MD, *Vienna, Austria*

Reply from the Authors: We thank Dr. Jellinger for his interest in our article.[1] His results provide support for our conclusion that the degree of concomitant neuritic AD pathology in DLB has an important influence on the clinical characteristics and, therefore, the clinical diagnostic accuracy of DLB. As in our study, Dr. Jellinger finds relatively low overall clinical diagnostic accuracy for DLB. He also reports very similar accuracies for patients with low (70% his vs 75% ours) compared to high (22% his vs 38% ours) Braak stages and low prevalences for at least some of the core clinical features of DLB in his samples. This is particularly interesting in light of the fact that our cohorts differ somewhat pathologically with regard to Braak stage stratification. However, the greatest difference in our cohorts is likely clinical because, as a dementia referral center, the initial presenting symptom in all of our subjects was dementia.

Despite this, and consistent with previous studies,[6,7] it appears that the prevalence of core clinical features in DLB is low and that, not surprisingly, clinical diagnostic accuracy remains poor. In our study, clinical diagnostic accuracy of DLB improved when core clinical features were present. We found that DLB subjects with high Braak stages were less likely to express the clinical features of DLB, making its recognition, and differentiation from AD, more difficult. We believe that our results and those of Dr. Jellinger emphasize the need for better diagnostic tools for DLB to ensure improved identification of these individuals in life.

Jody Corey-Bloom, MD, PhD, Annette R. Merdes, MD, Lawrence A. Hansen, MD, *La Jolla, CA*

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Severe intoxication after phenytoin infusion: A preventable pharmacogenetic adverse reaction

To the Editor: We read with interest the report by Citerio et al.[1] describing the genetic profile of a patient with presumed phenytoin (PHT) slow metabolism. We are concerned that the plasma concentration/time data provided by the authors do not support the hypothesis of the patient truly having slow PHT metabolism phenotype. Alternate explanations for these data are more likely.

First, a plasma concentration of 79 mcg/mL within 2 hours of a 15 mg/kg PHT loading dose primarily reflects dose size and volume of distribution, and only minimally reflects rate of metabolism.[2] The data presented suggest that the patient had a very small volume of distribution, a laboratory error, or, more likely, that the patient received an incorrect dose of PHT. Second, a patient with a typical PHT metabolic rate would require 8 days for the plasma concentration to drop from 79 to 10 mcg/mL based on data generated from a Bayesian PHT dosing program we have tested extensively.[3,4] We entered the patient data (extrapolated from the graph provided) presented by the authors into our modeling program and estimated a Km of 5.8 and a Vmax of 411, very close to the population-based estimates for a 75-kg 41-year-old woman (Km 5.7, Vmax 432). We modeled the plasma concentrations for a 41-year-old 75-kg woman who received a 3,000 mg PHT loading dose, and the graph closely approximates that shown by the authors, requiring 8 days to reach a level of 15 mcg/mL.

We are unconvinced that this patient had a slow PHT metabolizer phenotype and believe an incorrect loading dose with near normal PHT pharmacokinetics is a more likely explanation for the findings.

Michael D. Privitera, MD, *Cincinnati, OH*;
Timothy Welty, PharmD, *Birmingham, AL*

To the Editor: We read with interest the article by Citerio et al.[1] In their article, the authors indicated that a mutation of the CYP2C9 gene (CYP2C9*1/*3) was responsible for the high PHT levels (79 μg/mL) immediately after IV loading (15 mg/kg). We think that the patient's elevated PHT levels were not related to the CYP2C9 mutant allele.

The computed volume distribution (Vd) of this patient is 0.19 L/kg (Vd = dose [mg/kg] divided by serum concentration [mg/L]). The Vd of PHT in humans ranges from 0.7 to 1.2 L/kg,[5] and it is unlikely that the patient had a Vd of 0.19 L/kg. If the patient had an average Vd of 0.7 L/kg, she must have received approximately 55 mg/kg of PHT to achieve a peak concentration of 79 μg/mL. In other words, the patient received an excessive dose of PHT accounting for the very elevated PHT levels. The peak PHT levels are not related to the clearance or metabolism of a drug.

The CYP2C9 enzyme is only related to phase I metabolism of PHT. This patient's initial PHT intoxication is unrelated to the CYP2C9 gene mutation.

It is also worthwhile to analyze further the probable effect of the CYP2C9*1/*3 genotype of this patient on the elimination half-life of PHT. The elimination half-life of PHT is concentration dependent, and longer at higher initial concentration.[6] The known Vmax value is 6.2 mg/kg/day (0.26 mg/kg/hour) and Km value is 10.4 mg/L with a CYP2C9*1/*3 genotype.[7,8] The apparent half-life (t 50%) can be calculated by fitting the integrated form of the Michaelis-Menten equation.[9] We can estimate that the patient's half-life would be 179 hours from 79 to 40 mg/L and 143 hours from 60 to 30 mg/L (see Appendix). However, in the article, the PHT level dropped from 79 mg/L to about 40 mg/L in approximately 4 days (96 hours) and from 60 mg/L to approximately 30 mg/L in approximately 2 days (48 hours).[1] It is probable that the activated charcoal could have accelerated the elimination of PHT in this patient, resulting in relatively faster PHT elimination than the expected values mentioned above.

Appendix

Integrated Michaelis-Menten equation:

$$t\ 50\% = (0.5 \times C_i + Km \times In2)/Vmax$$

In2 is the natural logarithm of 2 (0.693)

Ci (initial concentration)

$$t\ 50\% = [(0.5 \times 79) + 10.4\ (0.693)]/0.26 = 179 \text{ hours}$$

$$t\ 50\% = [(0.5 \times 60) + 10.4\ (0.693)]/0.26 = 143 \text{ hours}$$

David V. Lardizabal, MD, Hans O. Lüders, MD, PhD, Collin A. Hovinga, PharmD, *Cleveland, OH*; Blaise F.D. Bourgeois, MD, *Boston, MA*

Reply from the Authors: We read with interest the letters by Lardizabal et al. and Privitera and Welty on our recent report about a PHT intoxication case associated with mutation of the CYP2C9 gene (CYP2C9*1/*3).

Both letters raise similar concerns, which can be summarized as follows:

1. High first PHT plasma concentration, after the bolus injection, probably unrelated to the genetic defect
2. PHT drop in the following days speedier than expected
3. Causative association with mutation of the CYP2C9 gene

1. In the dramatic initial scenario we described in the report, our initial hypothesis was the association of the clinical picture with the recently infused PHT. The suspicion of an error in the PHT delivered dose was, however, rejected after checking the nurse's record, checking the pharmacy consumption, and directly interviewing the staff on duty that night. Anyway, this kind of error can certainly be excluded because in Italy only one PHT injectable preparation (50 mg/mL/5 mL vial) is available. The patient received three vials for a total dosage of 750 mg of PHT. We agree with the pharmacokinetic calculations reported by Lardizabal et al. and Privitera and Welty, but we would like to point out that they apply to healthy subjects, whereas the patient we describe was severely ill.
2. The PHT levels fell faster than expected if calculated according to the Michaelis-Menten equation. To obtain faster PHT elimination, we used activated charcoal. This strategy was effective.
3. Association does not mean cause. In the article, we stated that a genetic polymorphism of the CYP2C9 gene "may be responsible for the toxic PHT levels."

We were only able to document an association with a genetic polymorphism of the CYP2C9 gene. There may be some other explanation but we have no evidence and have to limit ourselves to the information we have.

Giuseppe Citerio, MD, *Monza, Italy*; Alessandro Nobili, MD, *Milan, Italy*

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