Anorexigen-Associated Pulmonary Arterial Hypertension and the Serotonin Hypothesis: A Story Worth Telling

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SEROTONIN HYPOTHESIS
The serotonin hypothesis of pulmonary arterial hypertension (PAH) was first proposed in the 1990s after small studies in primary pulmonary hypertension (PH) found increased plasma serotonin levels and abnormal platelet serotonin storage.1,2 (Throughout this review, primary PH [PPH] is used when it was used in the original manuscripts; in most cases this refers to what is now called idiopathic PAH [IPAH], although in some studies drug/toxin-associated PAH was also included under this term.)

The serotonin hypothesis gained popularity after case-control studies suggested an association between anorexigen and PPH. Several serotonin-related mechanisms were suggested to explain this association.3-6 First, anorexigen increase free serotonin levels, activating serotonin receptors and causing pulmonary artery vasoconstriction and pulmonary artery smooth muscle cell proliferation. Also, norfenfluramine, a fenfluramine metabolite, has direct activity on the serotonin 2B receptor.7 Finally, internalization of the drug via the serotonin transporter may disrupt internal serotonin storage and promote pulmonary artery smooth muscle cell (PASMC) proliferation.

The serotonin story was not without controversy, however. A few studies questioned the role for serotonin signaling in the association between fenfluramine and PAH, because serotonin levels were lower after chronic fenfluramine exposure in animals. However, subsequent more careful measurements described a 2- to 4-fold increase in serotonin levels.8,9 The authors of these studies suggested that the prior results were due to difficulty in preventing serotonin release during blood handling and a real reduction in intracellular serotonin stores after long-term exposure. (Of note, similar issues may have contributed to subsequent variability in free serotonin levels in studies of PAH in patients as well.10)

Over the years, the serotonin hypothesis has been revised and expanded, but remains generally intact (Figure 1). Modern work has emphasized the importance of local serotonin production and signaling,11 the serotonin 1B receptor (vs other serotonin receptor subtypes),12 and interactions between estrogen pathways and serotonin signaling.13

CLINICAL STUDIES FOCUSED ON SEROTONIN SIGNALING
A number of medications targeting serotonin receptors have been investigated in clinical studies in patients with PAH, with disappointing results thus far. This includes hemodynamic studies of serotonin 2A and serotonin 2A/2B receptor antagonists (Table 1). Subsequent studies suggest that the

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serotonin 1B receptor is more important in pulmonary vasoconstriction in human lungs, but clinical studies are lacking. The serotonin transporter has also been considered a potential therapeutic target, based on both in vitro human and animal studies and in animal models of PH due to monocrotaline or hypoxia. We recently completed a small open-label study of fluoxetine in PAH (N = 10), and found no significant hemodynamic improvement or worsening over 12 to 24 weeks. Other researchers in this field have suggested that a more effective strategy could be to target both serotonin receptors and the serotonin transporter at the same time. Combined 5-HT1B receptor-serotonin transporter antagonists are effective in preventing and reversing experimental PAH and at reducing serotonin-induced proliferation of PASMCs derived from IPAH patients. However, it remains to be seen whether the combination of 5-HT1B receptor/serotonin transporter antagonists will have similar effects in clinical studies.

### STIMULANTS: SEROTONIN VS OTHER MECHANISMS OF ACTION

Most prescription and illicit stimulants as well as the diet pills aminorex, fenfluramine, and dexfenfluramine act as monoamine transporter substrates. Cocaine, the major exception, is a monoamine transporter reuptake inhibitor. Transporter selectivity varies considerably. All 3 diet pills act as serotonin transporter substrates. Methamphetamine, and to a lesser extent amphetamine, also have activity at the serotonin transporter. Cocaine, the major exception, is a monoamine transporter reuptake inhibitor. Transporter selectivity varies considerably. All 3 diet pills act as serotonin transporter substrates.

Table 1. Hemodynamic Studies of Medications Acting on Serotonin Signaling in Humans

| Target                  | Patient Population | Results                                      |
|-------------------------|--------------------|----------------------------------------------|
| Ketanserin59            | 5-HT2A receptor antagonist | PAH                                          |
|                         | PVR fell 12% vs baseline (P<0.001); SVR fell 16.5% (P<0.001) | Acute hemodynamics only                       |
| Terguride (abstract)50  | 5-HT2A/2B receptor antagonist | PAH                                          |
|                         | No change in PVR vs placebo (16 weeks, terguride vs placebo: -0.5 Wood units, P=NS) |                                           |
| Fluoxetine (abstract)17 | Serotonin transporter reuptake inhibitor (SSRI) | PAH                                          |
|                         | No change in PVR vs baseline (12-24 weeks) |                                           |
| Sumatriptan13           | 5-HT1B/D receptor agonist | Healthy controls                             |
|                         | mPAP increased 58% acutely (from 16 mm Hg to 26 mm Hg) |                                           |
however, that evidence to support an association with these medications is relatively weak. Only phenylpropanolamine, found to be associated with PPH in one case-control study, has even been considered “possibly associated” in prior consensus documents.

In contrast, phentermine was included in 2 case-control studies, neither of which found an association, and only case reports have described phentermine- and methylphenidate-associated PAH. Some researchers have also argued that these case reports likely represented IPAH rather than drug-associated PAH, given the widespread use of these medications and small number of reports. Further studies will be needed to address these possible associations.

For the illicit stimulants, a number of other mechanisms could also contribute, as described in more detail in the Stimulants and PAH article in this issue by Ramirez et al. In addition to mechanisms related to the primary (intended) drug, contaminants and adulterants must also be considered. Some illicit drug users inject or inhale crushed tablets, and the talc used as a filler and lubricant for the pills may result in foreign body granulomas in the lung parenchyma or vasculature, contributing to PH. Another potential contributor has been described with regard to levamisole, an adulterant commonly found in cocaine. Levamisole is an antihelminthic agent used to “cut” cocaine, presumably because it is both relatively inexpensive and has some stimulant properties of its own. However, levamisole is associated with a number of serious adverse reactions, and, most notably, is also metabolized to aminorex. This adulteration could contribute to a resurgence of aminorex-induced PH.

It also remains unclear why some patients develop PAH after anorexigen exposure, but most do not. One hypothesis is that the anorexigen may serve as a “second hit” in patients who have a genetic predisposition to develop PAH. Indeed, patients with anorexigen-induced PAH are more likely to harbor BMPR2 mutations (9%). Moreover, serotonin increases susceptibility to PH in BMPR-deficient mice.

SELECTIVE SEROTONIN REUPTAKE INHIBITORS AND PAH: TO PRESCRIBE OR NOT IN PAH?

Selective serotonin reuptake inhibitors (SSRIs) block the reuptake of serotonin and cause increases in circulating serotonin levels as well as alterations in serotonin signaling in the central nervous system. This has raised questions about whether SSRIs could lead to serotonin-mediated pulmonary vasoconstriction. However, circulating levels appear to return to baseline during long-term administration.

Interestingly, the association between SSRIs and PH also appears to be different for newborns vs adults. A rat model showed that prenatal exposure to fluoxetine induces fetal PH. Furthermore, epidemiology studies reveal that risk for persistent pulmonary hypertension of the newborn (PPHN) is increased in neonates whose mothers report SSRI exposure. Although variability has been reported for this finding, meta-analyses and larger well-designed population studies have convincingly shown a small but statistically significant increased risk of PPHN with SSRI use late in pregnancy (after 20 weeks’ gestation).

In contrast to the neonatal studies, SSRI use has not been clearly linked with the development of PAH in adult studies. Four studies have investigated antidepressant use in PAH (all antidepressants combined or specifically SSRIs). Three utilized a traditional case-control methodology, none of which found a positive association between antidepressant or SSRI use and PAH. In fact, SSRI use was numerically lower in PPH patients compared with controls (P=NS or not reported in all three). A fourth study using an administrative dataset found a significant association with SSRI exposure (OR 1.6, 95% CI 1.1 to 2.1), but the authors themselves suspected residual confounding may have accounted for their findings.

A separate concern regarding the validity of this finding is that the accuracy of the PAH diagnosis code in database studies is often poor. Outcomes among PAH patients who are subsequently treated with an SSRI have also been investigated. These studies had mixed results, with improved outcomes in 2 studies, similar outcomes vs untreated patients in one study, and worse outcomes for the SSRI-treated patients in a fourth study. Finally, animal studies have found a protective effect in both monocrotaline- and chronic hypoxia–induced PH in adult rats and mice.

Given the absence of randomized controlled clinical trials of SSRIs in PAH, a firm conclusion is not currently possible. So practically, what recommendations can be made with regard to SSRI use in PAH? Unfortunately in adult PAH patients, depression is very common (25% prevalence) and SSRIs are considered a first-line medication for treatment of major depression. Although consensus statements are lacking, our current practice is to consider use of an SSRI for moderate or severe depression in PAH patients in whom they are otherwise indicated. For pregnant women, stronger evidence of harm has been suggested. Nevertheless, consensus documents from the American College of Obstetricians and Gynecologists (ACOG) currently note that “untreated maternal depression is associated with increased rates of adverse outcomes (eg, premature birth, low birth weight, fetal growth restrictions, postnatal complications), especially when depression occurs in the late second to early third trimester.” They recommend the initiation or continuation of SSRIs in the setting of past or current moderate or severe depression. Thus while we express caution, benefits of treatment may in many cases outweigh the risks of untreated depression. Further studies are required in this area.

ANOREXIGENS AND PAH: REVIEW/NEWER AGENTS

As much of the world continues to struggle with the obesity epidemic, anorexigen continue to be prescribed to aid weight loss, generally in combination with lifestyle approaches. Medications for obesity have a long history of controversy. Between the 1940s and the 1970s, amphetamines were commonly prescribed for weight loss. With recognition of the potentially addictive nature of amphetamines, the Bureau of Narcotics and Dangerous Drugs (the forerunner to today’s Drug Enforcement Agen-
imposed more restrictions on their prescription, and a shift in prescribing patterns toward alternative agents began.\textsuperscript{48} Fenfluramine and dexfenfluramine subsequently became the most commonly prescribed diet pills, prescribed alone and in combination with phentermine until their withdrawal from the market in 1997. Finally, benfluorex, which is molecularly similar to fenfluramine, was introduced in 1976 for the treatment of diabetes and metabolic syndrome. Although withdrawn from several European markets due to a suspected association with valvular heart disease, it remained available in France until 2009, when additional studies found an association with both valvular heart disease and PAH; an estimated 300,000 patients were exposed annually.\textsuperscript{49-53} Figure 2 shows a timeline of anorexigen approvals.

**Newer Agents**

Among the diet pills shown in the timeline in Figure 2, only phentermine remains available by prescription for this indication. However, a number of other newer anorectic agents and combinations of agents are currently available. This includes orlistat (pancreatic lipase inhibitor), liraglutide (glucagon-like peptide agonist), lorcaserin (5-HT2C agonist), bupropion-naltrexone, and phentermine in combination with topiramate. None have been significantly associated with PH, and one study of liraglutide found that it could prevent and reverse monocrotaline-induced PAH in rats.\textsuperscript{54}

Lorcaserin, as a 5-HT2C agonist, has been of particular interest to the PH community because it interacts with a serotonin receptor. In 2 randomized control trials including 3182 patients and 4008 patients treated for one year, no increase in cardiac valvulopathy or PH was seen in association with lorcaserin use.\textsuperscript{55,56} The size and duration of newer anorexigen studies has been mandated by the Food and Drug Administration, with goals of allowing assessment of both longer-term efficacy as well as adverse effects.\textsuperscript{57}

In contrast to the large studies described above, a larger study investigating the combination drug naltrexone-bupropion was terminated early related to premature disclosure of results from an interim analysis. Although no cardiovascular safety concerns were raised and no association with the development of PAH was found, completed long-term studies are lacking.\textsuperscript{58}

**CONCLUSION**

Anorectic agents like aminorex, fenfluramine, and benfluorex are strongly associated with development of PAH, while evidence in support of methamphetamine, amphetamine, and cocaine is growing. Current evidence suggests that alteration in the serotonin pathway by these drugs may play a major role in development of PAH. Since most exposed individuals do not develop PAH, genetic susceptibility and other factors are thought to play an important role. Serotonin receptors and transporters have also been considered as potential therapeutic targets. Studies so far have been disappointing, but a number of additional serotonin pathway targets exist. Potential future considerations include inhibition of serotonin synthesis, antagonists to the serotonin 1B receptor (vs antagonists to the serotonin 2A and 2B receptors), and targeting one or more serotonin receptors while also blocking the serotonin transporter.\textsuperscript{14}

**Figure 2:** Timeline of anorexigen approvals.

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