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Levamisole, Aminorex, and Pulmonary Arterial Hypertension: A Review

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Context: An epidemic of aminorex-associated pulmonary arterial hypertension (PAH), resulting in multiple fatalities, occurred in the 1970s ending only after the drug was removed from the market. In 2009, when it was found that horses de-wormed with the anthelmintic levamisole, metabolized that drug to aminorex, the same drug that caused the 1970s outbreak of PAH. The discovery would not have been cause for concern except to horseracing enthusiasts, who feared horse doping. However, at about the same time the relationship between levamisole and aminorex was discovered, cocaine cartels began adulterating cocaine with levamisole. The rationale for adulterating cocaine with levamisole remains to be established. The purpose of this short review article is to discuss possible reasons for levamisole contamination, new discoveries about the human pharmacokinetics of aminorex, and the possible relationship between aminorex and pulmonary arterial hypertension in man.

Evidence acquisition: Medline contents, as well as estimates published by the U.S. drug enforcement agency (DEA), US state department, and the European monitoring center for drugs and drug abuse (EMCDDA) were reviewed and relevant articles retrieved. There is uniform agreement among the various monitoring agencies analyzed.

Results: Approximately 70 percent of the U.S. cocaine supply and 40 percent of the European cocaine supply is contaminated with levamisole. Aminorex has the same binding affinities for serotonin and other neurotransmitters as amphetamine. As serotonin is considered to be an important factor in the development of PAH, the possibility of another epidemic of aminorex-induced PAH, this time among cocaine abusers, seems real, and threatening.

Conclusions: The results of the first human pharmacokinetic studies of aminorex were first characterized in 2013. The results suggest that while humans could produce aminorex from levamisole, they probably do not convert very much. Even though cases of PAH have been reported in cocaine users, the latest pharmacokinetic studies suggest that very little aminorex is actually produced from the ingested levamisole, probably not enough to cause PAH. However, since both cocaine and aminorex can cause PAH the situation remains unclear. the lack of a clear answer is partially the development of an insidious pulmonary hypertension, producing subtle and non-specific symptoms in its early stages.

Keywords: Levamisole; Aminorex; Cocaine; Amphetamine; Serotonin; Bone Morphogenetic Protein

1. Context

The pressure in normal human pulmonary arteries varies between 8 and 20 mmHg at rest, and may reach 30 mmHg or more during physical activity. Pulmonary artery pressures greater than 25 mmHg at rest are considered pathological. Under older classifications, pressures over 30 mmHg during exercise were considered abnormal, but that assumption has been proven incorrect and accordingly dropped from the world health organization's (WHO) classification of pulmonary hypertension, which is now based on the etiology of hypertension. Five broad categories of pulmonary hypertension are recognized. Group I constitutes the largest category, and it is the only category where plexiform (a characteristic vascular change of pulmonary arteries in pulmonary hypertension) lesions, are seen (1). The other conditions in Group I are shown in Box 1. Many of the disorders that comprise this class, such as connective tissues like scleroderma, infectious diseases like HIV, and congenital heart disease will be familiar. However, the connection with drugs and toxins is often overlooked and poorly understood.

Box 1. Types of Pulmonary Arterial Hypertension, Group I [a]

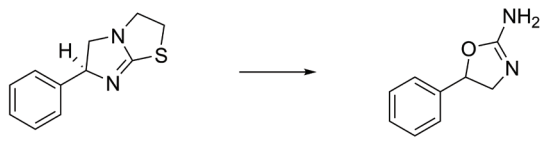
Pulmonary Arterial Hypertension
Idiopathic
Heritable
Drugs and toxins induced
Associated with
a. Connective tissue disorders
b. HIV infection
c. Portal hypertension
d. Congenital heart disease
e. Schistosomiasis
f. Chronic hemolytic anemic
Persistent pulmonary hypertension of the newborn
Pulmonary veno-occlusive disease/ pulmonary capillary hemangiomatosis

[a] Adopted from the current world health organization criteria.

Regardless of classification, vascular obstruction occurs because of excessive endothelial proliferation and apoptosis, resulting in increased resistance of vascular cells, as well as inflammation, thrombosis, and vasoconstriction. Many different theories have been proposed to account for these changes (2). The newest and perhaps most interesting theory involved, suggests that mechanical dysfunction is a key component (3). The suggestion is interesting because other studies have shown that exposure to methamphetamine induces mitochondrial-dependent apoptosis (4), while other amphetamines, particularly MDMA impair animal mitochondrial function at clinically relevant concentrations (5). Aminorex, because it is another amphetamine, is likely to exert similar effects.

The drugs most commonly associated with PAH are anorectics and abused drugs such as cocaine (6, 7) and methamphetamine (8), abused stimulant drugs, and the most commonly prescribed anorectics, are all selective serotonin reuptake inhibitors (SSRIs) (9). Rothman was first to demonstrate, in 1999 that, like other drugs known to cause pulmonary hypertension, aminorex was a serotonin transporter substrate (10), although recent studies have raised questions as to whether the relationship between SSRI antidepressants and PAH is actually causal. One drug not considered a risk for PAH is levamisole, Although it should be. In 2009, it was demonstrated that horses' metabolized levamisole, a decades old anthelmintic, to aminorex, an amphetamine (Figure 1). Aminorex has the same reuptake blocking capabilities as any other amphetamine, and is sometimes used to dope horses.

Humans also occasionally abuse aminorex (11) and an association between aminorex abuse and PAH has long been suspected (12). In 2012, our group published a case report demonstrating that humans are capable of the same conversion as horses (13), Although the kinetics of this conversion are only now being clarified (14, 15). The fact that this conversion occurs at all raises the possibility that levamisole administration might, ultimately, result in human PAH. Our group described one cocaine abuser with PAH who had aminorex detected in his urine and hair (16). But, because cocaine can cause PAH in its own right, any causal relationship remains speculative. The aim of this review was to figure out the relationship between levamisole ingestion, its metabolization to aminorex and onset of PAH through the analysis of the latest studies published in scientific literature. It is very important for public health since levamisole is used as adulterant of cocaine, one of the widely spread drugs of abuse; for this reason, research should be more focused on this study.



Levamisole Aminorex

Figure 1. Levamisole and Aminorex Structures

2. Evidence acquisition

For this review, many scientific databases have been used in order to collect information about cocaine/levamisole/aminorex and PAH. Medline contents, as well as estimates published by the U.S. drug enforcement agency (DEA), US State department, and the European monitoring center for drugs and drug abuse (EMCDDA) were reviewed and relevant articles retrieved.

3. Results

In 2004, levamisole was first detected in cocaine seized in the United States (17). Four years later levamisole made its European debut when 28 kilograms of cocaine hydrochloride, containing nearly 10 percent levamisole were confiscated in Rome. Late in 2007, cocaine contaminated with 8 to 20 percent levamisole began to appear in Colombian cocaine shipments destined for entry to the U.S. wholesale market. In 2008, the U.S. drug enforcement administration (DEA) published an analytic method for levamisole detection (18). The method was used to gather data for the DEA's Signature Program (a federally sponsored program that tracks the composition of cocaine seizures within the United States). In 2008, the DEA found that fewer than 10 percent of samples tested contained levamisole. By 2009, that number had risen to approximately 71 percent, where it is still thought to have remained, although recent reports from Europe suggest that a shift to adulteration with dexamisole, the levamisole isomer, dexamisole, may be occurring (19). The percentage of levamisole adulteration does not seem to have changed greatly since this adulterant was first introduced in the market.

Because levamisole was found in shipments entering the U.S., it is reasonable to assume that cocaine producers are responsible for adding levamisole to cocaine intended for export. This notion is supported by a recent report from Brazilian authorities who found that levamisole, in concentrations ranging from 0.7 to 23 percent, was the most abundant adulterant found in cocaine intended for shipment out of country (20). Why the drug trade should have adopted levamisole remains a mystery, but some think the purpose is to enhance the effects of the cocaine (21).

It has been suggested that cocaine and levamisole act synergistically (22), and that adding levamisole causes increased peripheral sympathetic activity and increased central neurotransmission, enhancing the intense feelings of pleasure produced by the drug. Recent experimental evidence argues against this theory (23). In vitro studies have shown levamisole itself has only moderate effects on neurotransmitter transporters, but that aminorex directly exerts potent psychostimulant effects. In fact, aminorex exerts strong effects on all three neurotransmitter transporters in a manner comparable to amphetamine (23).

Levamisole could be considered as a pro-drug, as it only exerts CNS effects after it has been converted to ami-

norex. However, elsewhere in the body, levamisole exerts toxicity in its own right. Users of levamisole-adulterated cocaine users are presenting at emergency rooms in increasing numbers. Three disorders, which in the past were relatively uncommon and almost unheard of in drug users have become very common: agranulocytosis, ANCA necrotizing vasculitis, and cutaneous necrosis (24-26). As aminorex is a known cause of pulmonary hypertension (27, 28), one might expect an increase in cases of pulmonary hypertension as well, but that has not proven to be the case.

Based on clinical studies of patients presenting for emergency room care, it can be estimated that an average symptomatic cocaine user has consumed more than a gram of cocaine per day. If the cocaine consumed actually contained 10 percent levamisole, roughly 100 mg of levamisole would have also been ingested. The result of this calculation is disturbing and perplexing because, among those who died during the aminorex epidemic of the late 1960s and early 1970s, the average patient was consuming far less than 100 mg of aminorex per day. The explanation is simple: humans simply do not metabolize very much levamisole to aminorex. Even if a heavy cocaine user consumed 8-10 grams of levamisole-contaminated cocaine per day, and they do (29), they still would only be ingesting trivial amounts of aminorex, simply because the conversion rate of levamisole is so low (15).

Elevations in plasma 5-HT (serotonin) have been implicated in the pathogenesis of both cardiac and pulmonary disease in methamphetamine abusers. Under normal circumstances, plasma 5-HT concentrations are kept at low levels by transporter-mediated uptake of 5-HT into platelets, and also by metabolism to form 5-hydroxyindoleacetic acid (5-HIAA). Many abused drugs, including amphetamines, target 5-HT transporters and, indirectly, increase the amount of circulating 5-HT. Animal studies have shown that drugs like amphetamine, methamphetamine, and aminorex, raise 5-HT levels sufficiently to initiate mitogenesis in pulmonary artery smooth muscle cells, thereby activating the signaling cascade that ultimately leads to PAH (30) and ultimately the same histological changes seen in man (31). Mounting evidence indicates that elevated 5-HT concentrations also account for the smooth muscle hypertrophy that is always evident in the hearts of chronic stimulant abusers (32).

Type I pulmonary arterial hypertension is characterized by the formation of plexiform and concentric lesions composed of proliferative vascular cells. As the process advances, there is increased pulmonary vascular resistance, leading to right ventricular failure and, if left untreated, ultimately death. In experimental animals, when PAH occurs, intimal and medial hypertrophy of the precapillary pulmonary vascular system occurs. Ultimately, these changes lead to vessel obliteration, in situ thrombosis, and plexiform lesions that, taken together, constitute the primary lesions of PAH.

The confluence of all these changes is referred to as vascular remodeling, and the etiology is clearly multifactorial. The most consistent finding in PAH is medial hypertrophy, a consequence of some, as yet uncharacterized, abnormality within the pulmonary artery smooth muscle cells themselves. The details of this process are far too complex for this short review, but genetic studies point to the involvement of bone morphogenetic protein (BMP) receptor type II gene (BMPR2). Somehow, mutation in the BMPR2 gene controls and/or initiates the vascular remodeling process within the pulmonary vasculature. At the moment, attention is focused on the upstream role of microRNAs (33) (which control more than 60% of the human genome (34). These microRNAs act as posttranscriptional regulators for the expression of BMPR2.

Much of what we know about aminorex we learned from an outbreak of aminorex poisoning that occurred in 1967, not long after aminorex first became available on the commercial market in Austria, Switzerland, and West Germany. In January 1971, a brief report in the British medical journal described the sudden occurrence of a particularly virulent form of pulmonary hypertension. The etiology was undetermined, but the common denominator in all of these patients was the prolonged use of the new anorectic drug, aminorex fumarate.

The first cases of aminorex poisoning were reported six to 12 months after aminorex fumarate (sold under the brand names Minocel and Apiquel) came to market (35). The average dose per day ingested by the affected patients (mostly women) ranged from 14 to 42 mg/day; the average patient had been taking the drug for more than one year (28). A Swiss researcher reported that in 1968 alone, the number of patients presenting with pulmonary hypertension had increased 10- to 20-fold (36).

At the time of this outbreak, the science of pharmacokinetics was in its infancy, and very little was known about aminorex, even after it came to market. Now, thanks to the work of German researchers a good deal more is known. After giving 100 mg doses of levamisole to native volunteers, standard pharmacokinetics studies were performed (Table 1). Levamisole could be detected in serum for up to 36 hours after ingestion, as could aminorex, but at no time did aminorex concentrations ever exceed the limit of quantification (LOQ). Levamisole kinetics are best described by a one-compartment model. The following parameters were calculated: ka = 1.2 [1/h], CL/F = 52 l/h, V/F = 347 l, f (renal)= 0.0005, $t_{1/2}$ = 2.0 h, AUC = 1923 ng/mL*h, cmax = 214 ng/mL, tmax = 1.98 h [15]. Interestingly, it was also found that levamisole could be quantified in 42.5 percent of cocaine-positive plasma samples (range 2.2 to 224 ng/mL), but that aminorex was positive in only 11.3% of the cases and, as indicated above, never at levels higher than the LOQ (Table 1). Some have suggested that given that the half-life of aminorex is longer than that of cocaine, the intent of adulteration may be to prolong the "high" associated with cocaine use, and now that the pharmacokinetics of levamisole have been elucidated, the suggestion seems plausible.

Table 1. Human Pharmacokinetics of Levamisole [a]

Parameters	Value
ka	1.2 [1.2 h]
CL/F	52/L/h
VF	347.1
f (renal)	0.0005
$t_{1/2}$	2.0 h
cmas	214 ng/mL
AUC	1923 ng/mL*h
Tmax	1.98

[a] Adopted from Hess et al. (15, 37).

4. Conclusions

The final verdict on cocaine/levamisole/aminorex and PAHis still not in and should be investigated more. One confounding factor is that the diagnosis of PAH is notoriously difficult to make, and even when the diagnosis is made, it is usually delayed. Based on the epidemic of the 1970s, it would appear that, on average, daily ingestion of aminorex for at least a year is required. Mostly, PAH patients complain of exercise intolerance, chest pain, and shortness of breath. None of these symptoms are very specific. These are exactly the same complaints expressed by patients with left heart disease, or other types of lung disease; they also happen to be the most common causes of PAH (14). Based on the dosages reported in the 1970s, only severely addicted cocaine abusers would seem to ingest enough levamisole to be at risk. Given these limitations, concerns about a cocaine/aminorex induced epidemic are not very great. With this study we tried to chart a course towards a real understanding on PAH risk in cocaine addicted, hoping that new studies will be performed. Only time will tell.

Authors' Contributions

Study concept and design: Steven B. Karch; acquisition of literature data: Steven B. Karch; analysis and interpretation of literature data: Steven B. Karch and Elisabetta Bertol; drafting of the manuscript: Steven B. Karch, Elisabetta Bertol, and Fabio Vaiano; critical revision of the manuscript for important intellectual content: Steven B. Karch and Elisabetta Bertol; administrative, technical, and material support: Steven B. Karch, Elisabetta Bertol, and Fabio Vaiano; Study supervision: Steven B. Karch.

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