Intranasal Antifungal Therapy in Patients with Chronic Illness Associated with Mold and Mycotoxins: An Observational Analysis

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Abstract

Exposure to mycotoxin producing mold and mycotoxins can be associated with numerous adverse health consequences. We previously reported that patients with chronic illness frequently had a history of prior exposure to water damaged buildings (WDB) and mold. Additionally, the vast majority of these patients had mycotoxins present in the urine. We have postulated that the mycotoxin producing molds were likely harbored internally in the sinuses of these patients. In the present analysis, patients with chronic illness and a positive urine mycotoxin assay were treated with intranasal antifungal therapy, either amphotericin B (AMB) or itraconazole (ITR). AMB was associated with local (nasal) irritation adverse effects (AE) in 34

Index terms— toxic mold, mycotoxin, chronic fatigue syndrome, intranasal antifungal therapy.

exposure occurred many years prior to the mycotoxin testing and furthermore, many of these patients did not report recent or current exposure to a WDB or moldy environment. Despite the remote history of exposure, these patients remained chronically ill and demonstrated the presence of significantly elevated concentrations of mycotoxins on urine testing. The persistence of illness years after exposure as well as the presence of mycotoxins suggested that there might be internal mold that represented a reservoir for ongoing internal mycotoxin production, either continuous or intermittent.

Recently we described the concept that the nose and sinuses may be the major internal reservoirs where the mold is harbored in biofilm communities [5]. This presence of mold can lead to the generation of mycotoxins internally. Thus, treatments aimed at reduction or elimination of the mold/fungi in the paranasal sinuses could lead to clinical improvement and in these patients. Herein, we present and discuss our observations in chronically ill patients who were treated with intranasal antifungal therapy.

1 II.

2 Methods and Materials a) Patients

All patients discussed herein had previously been diagnosed with CFS, similar to the patient population described in our previous study of mycotoxins in CFS [4]. Additionally, all were positive on the urine mycotoxin assay for at least one of the mycotoxins mentioned above. The age range of the patients reported and female to male ratio was very similar to the patient population previously published, in which the age range was 15 -72 years and 75% of the patients were females [4].

The rationale for the treatment with intranasal antifungal therapy was outlined in our previous paper regarding the role of naso-sinus colonization with toxic mold [5]. The concepts relating to such therapy were discussed with these patients at the time of a clinic visit. In patients that wanted to proceed with therapy, a prescription was then sent to ASL Pharmacy (see below). The patients were typically seen in follow up within three to six months

after initiating therapy. All patients reported herein were seen at least once in follow up after they started therapy.

3 Introduction

here has been a growing body of scientific literature indicating that exposure to mycotoxin producing molds and mycotoxins may be hazardous to the health of occupants of WDB (homes, schools and places of business) [1]. Water-damaged environments contain a mixture of biocontaminants produced by both mold and bacteria [1]. Secondary metabolites of molds (e.g. mycotoxins) have been identified in a variety of building materials and respirable airborne particulates, most commonly in WDB [2,3].

4 T

Using a sensitive and specific assay developed by RealTime Laboratories (RTL), we recently published a study linking the presence of aflatoxins (AT), ochratoxin A (OT) and/or macrocyclic trichothecenes (MT) to chronic fatigue syndrome (CFS) [4]. A significant number of these chronically ill patients were ill for many years, with average illness duration of more than seven years (range 2-36). Furthermore, over 90% of the patients gave a history of exposure to a WDB, mold or both. Exposure histories often indicated the WDB/ mold Institutional Review Board exemption was granted by Solutions IRB (Protocol #1FEB15-40). This was based on the fact that these patients were treated as part of their clinical management in the medical practice and not deemed to represent human subjects research.

5 b) Treatment

The therapy prescribed consisted of intranasal medications administered via an atomizer device. One agent was used to break up biofilm and the other an antifungal. Prescriptions were sent to ASL Pharmacy, Camarillo, California and then dispensed to the patients by ASL. The agents used to disrupt the biofilm consisted of a combination of ethylenediaminetetraacetic acid (EDTA) and a surfactant (polysorbate 80). Hereafter we will refer to that combination as the chelating agent (CHE). The CHE, which consisted of 2 milliliter (mL) of solution, was always given first, before the antifungal. The intranasal antifungal agents were either AMB or ITR. The AMB consisted of 5 mg in a solution of 3 mL. ITR consisted of 40 mg mixed in a solution of 4 mL. All intranasal applications were delivered via the Nasa Touch atomizer device provided to the patient by ASL Pharmacy. Patients generally administered the atomizer treatments once daily for each agent. The patients were advised to administer the CHE and respective antifungal separately (usually the CHE in the morning and the antifungal in the evening). Patients generally remained on therapy unless they discontinued it due to an AE. As discussed below, seven patients discontinued therapy unrelated to AE. The period of treatment observation ran for 12 months, May 2013 to May 2014.

6 c) Clinical Assessments

At the time of follow up clinic visits, each patient was asked to self-assess their improvement or lack thereof, that had occurred since starting therapy (compared to baseline symptoms before therapy). Improvements were categorized as: partial improvement (25% to 49% decrease in symptoms from baseline), moderate improvement (50% to 74% decrease in symptoms from baseline) or marked improvement (75% to 100% decrease in symptoms from baseline). The most common symptoms present at baseline and those commonly reported to improve on therapy were: fatigue, post-exertion malaise, body aching, headache and cognitive dysfunction. Since most patients had multiple symptoms, they were asked to make a global assessment as to whether they were overall improved from baseline and the degree (percent) of improvement. For purposes of the results reported in the Tables, the improvements (partial, moderate or marked) were grouped together. Thus, "improvement" represented at least a 25% or greater reduction in symptoms compared to baseline. Relapse was defined as recurrence of baseline symptoms after initial improvement.

At follow up, patients were also asked about AE that had occurred with the intranasal treatments. AE tended to be either local or systemic. Common local AE consisted of irritation symptoms in the nose and sinuses, to include: burning, congestion, nosebleeds, stuffiness, rhinorrhea and nasal/sinus pain. Systemic AE were always an exacerbation of baseline symptoms: fatigue (most common), headache, body aching and cognitive dysfunction. These were thought to be "die off" reactions (see below)

7 d) Mycotoxin testing

The urine mycotoxin testing of specimens were performed at RealTime Laboratories. The details of the assay have been previously described [4].

8 III. results

During the 12-month period of observation, 151 patients initiated therapy with CHE and AMB. An additional 14 were treated with CHE and ITR. The clinical results for each group are summarized in Tables 1 and 2. A subset of patients (n = 20) had repeat mycotoxin testing performed after several months on therapy. Of the 20

patients, 16 had been on AMB and 4 on ITR. Results of the repeat testing and clinical responses are summarized in Table 3. These patients continued on therapy, generally for greater than 6 months.

Additionally, seven patients, that had clinically improved, discontinued therapy (six from the AMB group and one on ITR). The most common reason given for discontinuation was that the patient felt as though they were probably "cured." These patients had repeat mycotoxin levels done while on therapy and another level after therapy had been discontinued. The data with regard to relapses and results of repeat mycotoxin levels after discontinuation of their treatments are seen in Table 4. In these patients, they had been on therapy at least 6 months when they discontinued the intranasal medication.

In summarizing the results from our patient observations, treatments with both AMB and ITR resulted in clinical improvement (reduction in symptoms).

In patients that used the AMB and remained on therapy without AE, 88 of 94 (94%) improved. Within this group, 26 of 88 patients (30%) graded their improvement as "marked" (defined above). We also found that AMB led to a decrease in the levels of mycotoxins in the urine assay. In the subset of patients on AMB (n = 16) that continued on therapy (generally at least 6 months) and had at least one repeat urine mycotoxin assay done, these repeat assays showed rather substantial and consistent decreases in the urine mycotoxin levels from baseline levels. AT (n = 4) and OT (n = 14) levels decreased in all cases tested and in all of these patients the levels dropped to zero. MT levels (n = 16) declined in 73%, albeit none dropped to zero. Several MT levels dropped rather dramatically, however, with levels as low as 0.01 ppb (data not shown).

Local AE in the nose and sinuses that resulted in discontinuation of therapy were common, seen in 34% of the patients on AMB. As noted above, systemic AE were not new symptoms, rather consisted of exacerbations of the patient's baseline symptoms. We felt these were most likely fungal "die off" reactions. These were frequently temporary, often lasting less than 3 to 4 weeks. However, in five AMB patients the systemic AE resulted in discontinuation. These systemic AE were not common, only seen in 13% of the AMB cases, albeit we suspect that these AE may have been under reported, given that a fairly high percentage of patients stopped therapy early due to local AE. AE that are reported with AMB, when administered intravenously, such as chilling, were not seen [6]. We did not see any systemic AE that were considered to be directly due to AMB [6].

ITR was quite effective, as well (albeit the numbers are much smaller). We noted clinical improvement in 80% of these cases. We also saw a decrease in mycotxin levels in ITR patients that had improved. Local AE were uncommon (less common that those seen with AMB). Systemic AE (presumably "die off") were seen with ITR but were uncommon.

We were also able to look at relapses in patients that had improved and elected to discontinue therapy. In seven patients that discontinued therapy (after improvement), six relapsed clinically (five on AMB and one that had received ITR). Most of these patients discontinued therapy around 6 months into the course of therapy. Furthermore, when mycotoxin levels were repeated after discontinuation of therapy (and relapse), the levels increased as compared to levels when on therapy (Table 4). OT levels increased after the patients stopped therapy in three of four cases. MT levels increased off therapy in four of four cases. When these patients resumed therapy (after discontinuation and

9 Discussion

Exposure to WDB, in particular, toxic mold, has been associated with numerous adverse health consequences [1,4]. We have studied patients with chronic illness, with the prototype being CFS. We found the chronic illness was highly associated with exposure to WDB/mold in the past and the ongoing presence of mycotoxins, detected with a sensitive and specific urine assay [4]. As we analyzed these patients, it became apparent that many of the patients with chronic illness and the presence of mycotoxins could trace their illness to past exposure but not recent or present exposure. We postulated that these patients may have harbored internal mycotoxin producing mold species and that such mold was likely in the sinuses, embedded in biofilm. A review of the literature and patient data supporting this idea was previously published [5]. Indeed, if these patients harbored mycotoxin producing molds/fungi in the sinuses, it seemed intuitive that therapies directed at reduction or elimination of this mold biofilm, could potentially lead to clinical improvements. Ponikau et al had previously found that fungi were very commonly found in the sinuses of chronic rhinosinusitis (CRS) cases **??**7]. This same group also showed that intranasal therapy with AMB had resulted in improvement in several clinical parameters in CRS patients **??**7]. Furthermore, AMB has been shown to be effective in fungal biofilm models [8]. Based on these types of data, we elected to offer treatment (intranasal AMB) to patients that were chronically ill (CFS) and had tested positive for mycotoxins.

We analyzed and report on 151 patients that initiated therapy with CHE and AMB, each administered once daily. Unfortunately, local AE in the nose and sinuses that resulted in discontinuation of therapy were common, seen in 34% of the AMB patients. These local AE were likely due to the irritation characteristics of AMB [6]. In patients that had minimal, if any local AE, the results were striking. We found that 94% of patients that continued on therapy (usually 6 months or longer) improved clinically. This was not particularly surprising given the prior published experiences with intranasal AMB in CRS cases, which frequently resulted in improvements in various clinical parameters (symptoms, endoscopic findings and computed tomography imaging results) **??**7]. Additionally, in our patients on AMB that improved and had repeat urine mycotoxin testing, we demonstrated substantial decreases in the urine mycotoxin levels from baseline levels. We have previously noted that repeat

urine mycotoxin levels in patients that were not on any type of therapy did not significantly change from baseline levels (unpublished observations). The decreases in mycotoxin levels in the patients on intranasal AMB showed a very good correlation with clinical improvements. Systemic AE (presumably "die off" reactions) were not common but may have been under reported, as noted above. We suspect, in the patients reported herein, that the systemic "die off" reactions were due to enhanced mycotoxin release when the therapy was initiated, as a direct result of the AMB interacting with the mold/fungi in the sinuses. In an in vitro model, Reeves et al demonstrated increased synthesis and release of gliotoxin from Aspergillus fumigatus upon exposure to amphotericin B [9]. Other than the local AE and "die off" reactions, AE directly attributable to AMB were not seen. Ponikau et al tested the sera of 3 patients for AMB in CRS patients treated with AMB and found no detectable drug **??**7]. Thus, it appears that AMB has no systemic absorption from the nose or sinuses.

We also studied intranasal ITR. Initially, we were concerned that it may be less effective due to the reports of poor biofilm activity [8]. However, we tried ITR as an alternative therapy in a small group of patients (n = 14). Despite the in vitro data regarding limited biofilm activity, when given along with the CHE, ITR was quite effective, as well (albeit the numbers were much smaller). Since ITR is orally bioavailable, it is potentially absorbed from the nose and sinuses in the setting of intranasal therapy. Albeit relatively small doses of ITR are used with intranasal therapy, there is the possibility of AE from the drug directly since we assume it could be absorbed systemically from the sinuses. Patients that had improved and discontinued therapy at approximately 6 months generally relapsed (six of seven patients). Furthermore, compared to the decreases in urine mycotoxin levels while on therapy, these levels increased after the patients had stopped their intranasal therapy. Thus, the duration of therapy remains a major question. Whether longer courses of therapy will be efficacious resulting in long term remissions remains unclear. It may be that some patients may need "maintenance" therapy to prevent relapses.

As stated earlier, the goal of intranasal antifungal therapy is reduction or elimination of the mycotoxin producing molds in the sinuses. From the data shown here, it appears that the mold levels in the sinuses can be reduced with intranasal therapy. It is unknown whether the mold can be eradicated.

V.

10 Conclusions

Despite the local AE (particularly AMB) and relapses when therapy was discontinued, the success rate with intranasal therapy was very encouraging. One major obstacle was the intolerance with AMB secondary to local AE. This analysis of intranasal antifungal therapy directed at mycotoxin producing fungi and biofilm in the sinuses, offers a very promising therapy alternative for patients with chronic illness associated with mycotoxins

11 Future Directions

There remain a number of unanswered questions with regard to intranasal antifungal therapy in these types of patients. The agent of choice, proper dose, frequency of dosing, most effective way to administer the therapy and duration of therapy have not been fully elucidated. In view of the frequent local AE with AMB, other antifungal agents need to be addressed. Certainly, ITR is one available option, however, the potential for systemic absorption is a concern, as noted above. Another option is intranasal nystatin. Although used for decades as a topical agent for yeast infections, nystatin actually has good in vitro activity for molds [10]. Since nystatin is a polyene antifungal agent (similar to AMB), it would be predicated to have similar effects. Hopefully, there may be less local AE due to nasal irritation. Additionally, nystatin is not systemically absorbed and has a long track record of clinical safety. Intranasal nystatin was not available when this study was done. It may be a potential option to pursue.

There is also interest in alternative agents to break up the biofilm. In that regard, mupirocin has been studied in CRS patients and has been an effective therapy [11]. Additionally, mupriocin appears to be active against biofilm [12]. It may represent an interesting agent to address for these types of patients in the future. [1]

Figure 1:

1

Group	Number	%
AMB Total Patients	151	100
AMB Clinical Response: Improved *	88	58
AMB Local AE Resulting in Discontinuation **	52	34
AMB Systemic AE Total (with or without Local AE) ***	19 ****	13
AMB Continued Therapy & Improved	88	94

*[Note: * Improvement defined in Methods section, ** Local AE defined in Methods section, *** Systemic AE defined in Methods section, **** 5 patients discontinued therapy due to systemic AE]*

Figure 2: Table 1 :

2

Group	Number	%
ITR Total Patients	14	100
ITR Clinical Response: Improved *	8	57
ITR Local AE Resulting in Discontinuation **	1	7
ITR Systemic AE Total (with or without Local AE) ***	3 ****	21
ITR Continued Therapy & Improved	8	80

*Improvement defined in Methods section, ** Local AE defined in Methods section, *** Systemic AE defined in Methods section, **** all 3 patients discontinued therapy due to systemic AE

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Figure 3: Table 2 :

3

Rx	Imp	%	AT dec	%	OT dec	%	MT dec	%	Total
AMB	14/16	88	4/4 *	100	14/14 *	100	11/15	73	16
ITR	3/4	75	1/1	100	3/4	75	3/4	75	4

*[Note: Rx: Treatment, Imp: improved, AT dec: aflatoxin level decreased, OT dec: ochratoxin A level decreased, MT dec: macrocyclic trichothecene level decreased, AMB: amphotericin B, ITR: itraconazole, *decreased down to a level of zero (AT 4/4, OT 14/14)]*

Figure 4: Table 3 :

4

Rx	Imp	%	Rela p	%	AT inc	%	OT inc	%	MT inc	%
AMB	6	100	5/6	83	n/a	n/a	3/4	75	4/4	100
ITR	1	100	1/1	100	n/a	n/a	1/1	100	1/1	100

[Note: Rx: Treatment, I: improved, Relap: clinical relapse after discontinuation, AT inc: aflatoxin level increased compared to level obtained on treatment, OT inc: ochratoxin A level increased compared to level obtained on treatment, MT inc: macrocyclic trichothecene level increased compared to level obtained on treatment, AMB: amphotericin B, ITR: itraconazole, n/a:not applicable]

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