

Pathology of mycotoxicoses: possibilities and limits of a diagnosis

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Abstract - The goal of necropsy is to gain a complete understanding of the dissected organism and the standard method for necropsies is essentially a reductive organological analysis of the pathological material. The pathologist is placed in the position of an intermediary between the basic sciences and the clinical sciences of medicine, and this demands from the pathologist to synthesize and to apply the totality of available scientific information to each case. No other discipline is charged with this responsibility, and it is the duty of the pathologist to present his final diagnosis at the level of organization where the investigation began: the total organism. The effects of various mycotoxins on various organs and systems of the body are to be considered first, and the specificity or non-specificity of a lesion with respect to a given or suspected mycotoxin has to be assessed. Unfortunately, many mycotoxins produce symptoms or lesions which do not allow for a diagnosis on the basis of singular observations, but when the lesions are considered in a holistic manner, many mycotoxins can be diagnosed accurately. Limitations are the non-specific nature of primary injury, which may be masked by secondary effects, and late-appearing effects (e.g., neoplasia) which may be attributed to other carcinogens. Further complications arise due to the interaction of two or more mycotoxins and/or toxicants, and species variability with respect to type and site of response is another problem. Finally, while modern analytical techniques may allow for determination of traces of metabolites in the body, the causal link may be difficult to establish because the primary source of the mycotoxin may no longer be accessible for investigation. Judicious use of all the knowledge that is available nowadays, however, should permit a much higher rate of diagnoses and would allow for widening of our understanding of these naturally occurring toxins.

INTRODUCTION

The goal of a post-mortem examination is to gain a complete understanding of the dissected organism which requires a standard necropsy method for what is essentially a reductive organological analysis of the pathological material. Since toxic properties of chemicals manifest themselves as functional, biochemical or structural changes, a documented functional change may give important clues as to where a structural change might be found. As for biochemical changes, these may or may not be accompanied by structural changes. To complicate matters further, there is only a limited number of ways in which damage may appear or can be expressed; a dead cell is a dead cell, without giving any clues as to what injury took place.

The frontiers of anatomical pathology lie at the interrelationship of one organ system to another and at the integration of knowledge derived from biochemical or other studies, which give insight beyond the morphologically defineable changes. This task places the pathologist into the position of an intermediary between the basic sciences of medicine and the clinical sciences of medicine, and demands from the pathologist to synthesize and to apply the totality of available scientific information to each case.

No other discipline is charged with this responsibility: The basic scientist, bound to his discipline, is restricted by either anatomical, biochemical, physiological or other reductive attributes of the organism; the clinician is bound to his area of specialization and interest. Thus, it is the pathologist who has to look at all the evidence and present the final analysis at the level of organization where the investigation began: the total organism. This is no easy task, and some consider this to be a problem. Yet, the real problem is: not having a pathologist who has a suspicion, therefore not even attempting to make a diagnosis and, instead, just listing some disconnected observations. Unfortunate as it may be, this is precisely the situation in which we find ourselves with respect to most mycotoxicoses, because many hold the view that mycotoxins are agents in search of a disease.

It is the purpose of this paper to consider the limitations and the possibilities of making a diagnosis. Before we enter into the specific discussion, though, it may be advisable to consider briefly a few principles.

Knowledge of the effects of a toxin, and this is no different with mycotoxins, stems from various sources, such as: disease outbreaks with subsequent intensive investigation; toxicological studies in which the toxin was given to animals, with subsequent examination of certain aspects - mostly depending on the investigators' particular expertise, interest, and preferences.

If one looks at the vast and voluminous literature on mycotoxins, one can categorize the publications and critically evaluate them in a somewhat liberal and arbitrary way. There are five groups:

Group 1: Includes case reports and clinicopathological observations of events where mycotoxin involvement was suspected; in a few instances, actual proof was eventually provided. This group contains reports that are interesting and important, but not highly regarded in the scientific community. They lack specificity, and the toxic principle is either completely unknown or only partially known, but they are, actually, the only descriptions of real events, thus extremely important.

Group 2: Includes reports on findings of fungi or mycotoxins in commodities, often or mostly without any direct linkage to a specific disease. These publications often appear to be not very valuable on a first look. Reports in this group may be labelled misleading, alarmist, disconnected - and yet they are the much needed pieces of a puzzle to complete the picture.

Group 3: Consists of reports on experiments in which the toxin or the suspected commodity, etc., was applied in the same manner as it is consumed naturally. Such publications provide the most valuable information: either the toxin was used (as purified compound) or feeding was done with commodities suspected to carry a particular mycotoxin, under controlled conditions. These studies provide the best insight into what the toxin is actually capable of doing, although they are not infallible either. The experiments were conducted, in all likelihood, under ideal conditions, thus not repeating the normal-life situation.

Group 4: Includes pathological reports of experiments in which toxins were applied in all sorts of unusual ways (i.v., s.c., i.p., etc.) or in extraordinarily high concentrations. Numerous pathological findings are listed in such publications, often seducing the reader to believe that the toxins used are omnipotent. On the other hand, these reports are regarded highly as a reliable source of information, yet they are misleading or are prone to be misinterpreted, because the effects seen are not truly representative of what may occur in the real world. Three examples should illustrate this point. Does it really help our understanding of aflatoxicosis when the LD₅₀ of aflatoxin is established using intraperitoneal application, "because it was not possible to obtain a reliable LD₅₀ after gavage, due to variation in the response of the animals" (ref. 1)? Is it justified to call zearalenone a mycotoxin with potential immunotoxic effects, because chickens given 1,600 ppm of zearalenone showed a reduction in the weight of the bursa of Fabricius (ref. 2)? What should be done with the observation that patulin is more toxic by s.c. and i.p. routes than i.v., but least toxic when given per os, the most likely route of consumption (ref. 3). Such reports do give some valuable insights into the mechanisms at work, but they should not be expected to serve as a guide for classification of a mycotoxin as being able to produce typical disease in one or the other organ.

Group 5: Consists of publications describing the results of biochemical, cellular-biological and other studies with toxins. As with Group 4, this group, again, allows for an inside-look at the mechanisms involved, but extrapolation to real life situations or disease has to be made with great care.

Some, if not most, of the uncertainties and hesitancy observed when it comes to making or accepting a pathological diagnosis of a mycotoxin-related disease stem from the fact that the observations and findings from all 5 groups are mixed ad libitum, thus obfuscating the really important aspects or distracting from the main objective. Group 1, in particular - the natural outbreak reports - provides a never-ending source of confusion by continuing to use catch-all terms like "fusariotoxicosis" or worse "mouldy grain disease" or "ill-thrift." Terms like "alimentary toxic aleukia" are a bit more helpful, in that they at least describe specific toxicopathological aspects.

Group 4 reports are the never exhausted source of many critical remarks about pathologists and their inability to transmit, to the non-pathologists, the important and salient features of their observations. No wonder, then, that we find in review articles correlations between a mycotoxin and a particular system of the body, based on a spurious, ambiguous, or secondary pathological observation, which might or might not be directly associated with the particular organ. One example may suffice: hydrothorax and ascites in cases of experimental

sporidesmin poisoning in rats (ref. 4) are signs of a generalized increased vascular permeability which could be associated with a number of principle organic lesions, e.g., in the heart (cardiac insufficiency), in the liver (protein synthesis) or the vessels themselves (increased permeability). It isn't very helpful if such a lesion shows up under a main heading: "effects on the cardiovascular system."

The best information is, of course, gained from a very careful selection of various components from all groups, using from each group only as much as is necessary to make a point. In all likelihood, description of such a disease will be associated with the name of the toxic principle, the name of the mycotoxin.

Finally, before I come to the discussion of the limitations and possibilities of a diagnosis, I should say that I take it for granted that a number of basic aspects have been delineated already before a diagnosis on the basis of pathological findings is made. These prerequisites, as I would call them, are:

- (1) The disease observed is not transmissible from one animal or human to another, being neither infectious nor contagious.
- (2) Treatment with drugs or antibiotics has had little effect on the course of the disease.
- (3) The outbreak is essentially seasonal or associated with a specific setting, either geographically or otherwise.

Whether the suspected foodstuff reveals presence of fungi or not is irrelevant, because we know that toxins can be in the food without visible presence of fungi.

LIMITATIONS TO MAKING A DIAGNOSIS

Admittedly, there are a number of factors which make it rather difficult to make a diagnosis, such as:

- (1) The lesion may be so non-specific that it doesn't give a clue as to the cause. Hepatic necrosis is a typical example: any number of toxins (e.g., Blue-green algae) can cause hepatic damage.
- (2) The effects of the mycotoxins may be masked by secondary effects which appear to be primary effects (e.g., bacterial or fungal or viral infection in the case of immunosuppression). The secondary effects are responsible for the fact that the possibility of an underlying immunosuppressive agent is overlooked by the pathologist. Also, the clinical signs may be secondary to the site of action of the mycotoxin, as the example of Reye's Syndrome in case of aflatoxicosis shows.
- (3) A lesion (particularly neoplasia) is so late in appearance that a causal relationship can't be established.
- (4) Interaction of several mycotoxins or even other toxicants or deficiency states may produce bizarre effects which are not typical of any one of the mycotoxins alone and hence are ascribed to something else or to nothing.
- (5) Extrapolation from one species to another is difficult, because the target organ and biotransformation characteristics of a particular mycotoxin not only varies between species but also strains.
- (6) The final, causal proof may never be established because the causative agent is no longer in the food/feed, and metabolites may no longer be found, either.
- (7) Analytical processes are complex, expensive, not always available and, unfortunately, not always repeatable.
- (8) The Arndt-Schultz law also applies, with mycotoxins in high doses resulting in toxicity, whereas lower doses may cause hormetic stimulation (ref. 5).

POSSIBILITIES OF MAKING A DIAGNOSIS

(Instead of individual references, the reader is referred to summarizing review books, e.g., refs. 3, 6, 7, 8, 9, 10, 11, 12 & 13).

A few mycotoxins produce lesions that permit a definitive diagnosis, namely, ergot alkaloids, sporidesmin, dicoumarol, slaframine and zearalenone.

The clinical and/or pathological findings in these cases are highly suggestive of the presence of the particular toxins, but it should be noted that epidemiological or analytical findings are needed to confirm the diagnosis.

A much larger number of mycotoxins, however, produce lesions that do not permit a definitive diagnosis by pathological means alone, but are highly suggestive of a mycotoxicosis. These include aflatoxin, sterigmatocystin, psoralens, the trichothecenes, ochratoxin, citrinin, citreoviridin, Penicillium islandicum toxins, rubratoxin, patulin, the tremorgenic mycotoxins, moniliformin and the cytochalasins.

In the following part, various body systems will be considered to see how a careful examination might give clues as to the toxic principle involved. The frequently used and intellectually appealing categorization into "hepatotoxic," "nephrotoxic," etc., mycotoxins is so unreliable and actually misleading that it will not be used in this presentation.

1. Integumentary System: The photosensitizing ability of the psoralens has been well documented for centuries, and the secondary photosensitization due to hepatic damage by sporidesmin is well known. The only other group of mycotoxins that have a primary skin-irritating property are the trichothecenes (refs. 14 & 15).
2. Central Nervous System: The CNS-action of ergot alkaloids, in addition to their vasoconstrictive property, is a century-old mycotoxic disease. Many mycotoxins cause tremors, convulsions or paralysis, such as the tremorgens (penitrem, paxilline, verruculogen, fumitremorgens), citreoviridin, roquefortine and cyclopiazonic acid, without clearly identifiable morphological changes. Trichothecenes cause emesis and general CNS-disturbance, as evidenced by the DAS (Anguidine) treated patients (refs. 16 & 17). Moniliformin causes encephalomalacia in equidae, but not other species.
3. Gastrointestinal tract: The trichothecenes are the major group of mycotoxins which cause lesions ranging from perioral dermatitis, stomatitis, esophagitis and gastritis to the characteristic radiomimetic lesions in the intestine (refs. 13, 14 & 18). Many other mycotoxins are loosely associated with "gastrointestinal lesions," but in almost all instances, it is not clear whether a diarrhea or other symptom observed is not associated with the antibiotic effect on the intestinal flora. Cyclopiazonic acid causes degeneration and necrosis of Langerhans cells in the pancreas (ref. 3).
4. Hematopoietic System. Suppression of hematopoiesis has been seen with ochratoxin (ref. 19), but the only group of mycotoxins that have a devastating effect on the hematopoietic system are the trichothecenes, which can cause complete atrophy of the bone marrow (ref. 14).
5. Immune System: Aflatoxin, ochratoxin and citrinin have been found to have immunosuppressive effects (ref. 20), in addition to trichothecenes (ref. 14). Such effects can be observed in lymph nodes, spleen, thymus and the bursa of Fabricus.
6. Hepatobiliary System: Aflatoxin is the best known and most studied mycotoxin, causing hepatic necrosis, cirrhosis and hepatic cancer. Most, if not all, other mycotoxins affect, in a non-specific manner, the liver in one way or another, and hence are not considered here further.
7. Urinary System: While many mycotoxins can affect the tubular system of the kidney in a non-specific way, the only mycotoxins with a truly specific action upon the kidneys are ochratoxin, citrinin and patulin (refs. 21, 22 & 23).
8. Cardiovascular System: This system can be affected in many ways, such as lipidosis of the myocardium in case of aflatoxin toxicosis (ref. 23), or increased vascular permeability with sporidesmin (ref. 4), but such findings are not characteristic or indicative of these toxins. The same holds for the trichothecene-related cardiovascular problems or the frequently observed hemorrhages, often associated with disseminated intravascular coagulopathy. Moniliformin, on the other hand, appears to cause myocardial degeneration (ref. 25).
9. Respiratory Organs: Although the respiratory tract may serve as a port of entry for mycotoxins, there are no specific lesions or symptoms known to occur with mycotoxins.
10. Endocrine Organs: Enlargement or necrosis of the adrenals is often described (e.g., with aflatoxin (ref. 1) or sporidesmin (ref. 26) and other mycotoxins, but it is doubtful whether this is a specific reaction. It is more likely a general, stress-related response.
11. Reproductive System: The economically important effects of the estrogenic mycotoxin, zearalenone, in the hog industry are well known; the trichothecenes can cause impaired

spermatogenesis, and the embryotoxic and teratogenic effects of many mycotoxins, such as aflatoxin, ochratoxin, patulin, rubratoxin and the trichothecenes are well documented (ref. 27). For other toxins, such as the ergot alkaloids (ref. 28), a relationship to the reproductive system is more suspected than proven.

CONCLUSIONS

A very critical evaluation, like the one attempted here, identifies apparently only a very few mycotoxins that cause characteristic lesions. Many of the often quoted relations such as "organ affected in such and such a way" do not seem to stand up to critical scrutiny. And yet, as said in the beginning, the frontiers of pathology lie at the interrelationship of one organ system to another and at the integration of knowledge derived from various other studies. This is where the real possibilities for exploration and further research lie. What was given as an example of a limitation, i.e., that action of more than one mycotoxin may cause a hitherto unexplained lesion, turns out to be one of the most challenging and promising areas of research. One good example is the attempt to explain the high esophageal cancer rate in some geographic locations, such as in the Transkei, where it appears that the synergistic action of three totally unrelated mycotoxins, deoxynivalenol, zearalenone and moniliformin (refs. 29 & 30) plus another mutagenic metabolite, Fusarin C (ref. 31) may cause this type of cancer. Another example is the synergistic action of various trichothecenes (ref. 32) which cause a more serious and more lethal disease than one compound alone.

A standardized approach of critical organological analysis of all pertinent findings, and a judicious use of all the knowledge that is available from other sources, which include epidemiological and clinical history data, should permit us to widen our understanding of the importance of naturally occurring metabolites, called mycotoxins, far beyond what we think now to be possible.

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