

Chemotherapy of Rhinosporidiosis: a Review

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ABSTRACT

Even though rhinosporidiosis was first identified in 1892, the published literature contain limited information on options for the chemotherapy of this disease. The absence of methods for in vitro culture of *Rhinosporidium seeberi* has restricted the development of in vitro drug susceptibility assays. A new method of 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H tetrazolium bromide (MTT) reduction was recently introduced to assess the viability of rhinosporidial endospores, the putative infective stage of *R. seeberi*. Using this modification for the microscopy of target endospores, eight antimicrobial agents have been found to be effective anti-rhinosporidial therapeutic agents (in order of decreasing potency: imidocarb dipropionate, diminazine aceturate, cycloserine, dapsone, trimethoprim-suphadiazine, ketoconazole, sodium stibogluconate, and amphotericin B). Fifty-percent inhibitory concentration (IC₅₀) values of less than 100 µg/mL were regarded as indicating therapeutic efficiency. In vitro determinations of the time-course of action of dapsone revealed more rapid inactivation than clinical responses suggested, probably because of the in vivo pharmacokinetics of dapsone that delay an access of the drug into the pathogen. While dapsone remains the best clinically documented anti-rhinosporidial agent, it is suggested that combination drug therapy may be advantageous, as in the treatment of tuberculosis, to forestall development of drug resistance by *R. seeberi*. The early use of anti-rhinosporidial medical therapy, especially in the absence of surgery and to preempt the dangerous complications especially in ocular rhinosporidiosis, is emphasized. (*J Infect Dis Antimicrob Agents* 2009;26:21-7.)

INTRODUCTION

Rhinosporidiosis was first observed in a human nasal polyp by Malbran in Argentina in 1892. Seeberi, also in Argentina, identified the pathogen and Wernicke named it *Rhinosporidium seeberi* in 1900.^{1,2} Rhinosporidiosis occurs in humans and in a

wide range of animal species, and has been reported from 70 countries. It is highly endemic in South Asia (India and Sri Lanka).

The major sites of rhinosporidiosis are the upper respiratory tract (the nasal cavity and the nasopharynx), the eye and its adnexae, the urethra especially in males,

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the skin, and rarely in disseminated sites including various viscera. The bone and cartilage are eroded, and the granulomatous polyps especially in the nasopharynx become very vascularized making surgical excision hazardous.

Spontaneous regression of rhinosporidial growths has been noted in animals and in humans but is rare, hence medical and/or surgical intervention is necessary. Radiotherapy has been shown to have no effect.³ The mainstay of treatment is surgical excision of the polyps, even though the recurrence is common. Since the early decades of the twentieth century, several drugs and proprietary preparations have been used, mainly in individual cases, the clinical outcomes have been either variable or inconclusive. A major problem with most of these 'trials' has been that the period of observation after surgery has been far too short. Other shortcomings have been unsuitable routes of administration such as topical application and a lack of histological studies of rhinosporidial tissues from treated patients except after dapson therapy.

Clinical reports on rhinosporidiosis still indicate there is a lack of information on the drug susceptibility of *R. seeberi*, and on effective anti-rhinosporidial chemotherapy. Data on drug susceptibility of other members of the Class *Mesomycetozoea* to which *R. seeberi* belongs, is also lacking.

Assessment of the susceptibility of *R. seeberi* to antimicrobial drugs

There is a contrast with the history of successful treatment of bacterial and fungal infections with medications after laboratory culture and other techniques. Similar methods for *R. seeberi* have not been possible. This is because *R. seeberi* is still not cultivable in vitro, and hence in vitro methods based on the multiplication of the organism have not been available. This problem was overcome when it was

found⁴ that the salt, 3-[4, 5-dimethyl-2-thiazolyl]-2, 5-diphenyl-2H tetrazolium bromide (MTT), could be used to assess the viability of the endospores of *R. seeberi*. This salt is used in lymphoproliferative assays⁵, and in studies of fungal viability after exposure to antimicrobial agents.⁶ Viable endospores of *R. seeberi* are reactive with MTT⁴, resulting in the formation, by MTT-reduction, of a deep purple-coloured formazan that stains the cytoplasm and some spherical bodies of the endospores. These bodies have been identified as the electron dense bodies (EDBs),⁴ that are extensively described in the literature on *R. seeberi*. Endospores have been found not to be capable of MTT-reduction after treatment with the standard microbial-inactivating agents, heat (100°C), 10 percent formal-saline, and sodium azide. Slow freezing to minus 20°C also rendered the endospores non-reactive with MTT.⁴ The MTT-reduction method has been adopted as a method for the determination of the viability of rhinosporidial endospores.

The method is straightforward. The endospores are incubated with various antimicrobials or other agents in aqueous suspension for selected periods at room temperature (28°C). The endospore specimens used are taken from freshly harvested surgical specimens of rhinosporidial nasal or nasopharyngeal polyps. If the inactivating agent under investigation contains serum, the treated-endospores are washed repeatedly to remove any serum that could inhibit MTT reduction⁷ before the MTT is added. The mixtures are incubated at 37°C for 3 hours, and the endospores (after brief, low-speed centrifugation) are examined under oil immersion at 1,000 magnification. This modification of the original MTT method eliminates the need for extraction of the formazan by acid-ethanol, as in lymphoproliferative assays, and its quantitation by spectrophotometry. The original method is liable to

cause spuriously elevated optical densities of the extracted formazan due to contamination microorganisms or even host's cells from the original nasal polyp.

MTT-reduction method depends on the action of dehydrogenases in the mitochondria in the cytoplasm of the endospores to produce the microscopically observable deposits of the deep-purple formazan; these bodies have been described in the cytoplasm but have apparently not been looked for in the EDBs which are capable of reduction of MTT. The advantages of the direct microscopic assessment of the intensity of MTT reduction by the endospores, rather than by spectrophotometric quantitation of extracted formazan, are (1) the intra-endosporial sites of MTT - reduction could be readily visualized microscopically as the cytoplasm and the EDBs, (2) inevitable contamination of the endospore suspension by nasal microorganisms and their resultant contribution to the formation of formazan is bypassed, (3) the method is simple and cheap, (4) it results in accurate estimation of the intensity of MTT reduction by counts of the endospores that have been morphologically damaged and that have been inhibited from reducing the MTT, moreover, the results from this in vitro MTT-reduction method, as distinct from conventional in vitro methods that depend on microbial culture, can be extrapolated to the in vivo situation by demonstrating a correlation between in vitro and in vivo tests.⁸⁻⁹

Susceptibility of *R. seeberi* to some antimicrobial drugs

Biocides

The modified MTT-reduction method has been used to assess the anti-rhinosporidial activity of 14 biocides (antiseptics and disinfectants) at concentrations used in hospital and laboratory practice¹⁰⁻¹¹, and also eight antimicrobial drugs with quantification of their 50-

percent inhibitory concentrations (IC₅₀).¹ These studies included several effective generically named anti-rhinosporidial biocides¹⁰ including hydrogen peroxide, glutaraldehyde, chlorohexenol, chlorhexidine, cetrimide, thiomersal, 70 percent ethanol, iodine in ethanol, 10 percent formalin, povidone-iodine, sodium azide, and silver nitrate. The effective seven brand-named biocides included¹¹ Bacillo-floor, Bactolin, Bodedex, Cutasept, Korsolex, Sokrena, and Sterilium. The extreme susceptibility of the endospores to biocides was striking, and the targets of their action were the intra-endosporial contents mainly the EDBs, and the endospore walls.

Drugs used in humans

Antimicrobial drugs that have been found to have significant in vitro anti-rhinosporidial activity (IC₅₀ of ≤ 100 $\mu\text{g/mL}$) include those used in human as well as animal treatment: amphotericin B, dapson, ketoconazole, trimethoprim-sulphadiazine, and sodium stibogluconate.¹ The IC₅₀s (with number of trials in parenthesis) of these drugs were (in decreasing order of potency) dapson 29.7 (10), trimethoprim-sulphadiazine 38.4 (9), ketoconazole 51 (8), sodium stibogluconate 55.7 (7), and amphotericin B 57.1 (8) $\mu\text{g/mL}$. The intra-endosporial targets were the EDBs, while the endospore walls remained unaffected except at very high concentrations. All five drugs were endospore-static rather than endosporicidal, and were non-lytic on the endospores. The two drugs for use in animals, berenil and imizol showed greater activity with IC₅₀ values of 13 (5) and 9 (1) $\mu\text{g/mL}$, respectively. It is noteworthy that berenil has also been used in human trypanosomiasis and babesiosis, and imizol in Lyme disease. Cycloserine, a drug used as anti-tuberculous medication, was recently found (Arseculeratne 2009, unpublished data) from 12 trials to have a mean (\pm SD) of 10.6 ± 5.95 $\mu\text{g/mL}$.

These in vitro results were correlated well with the data from clinical studies from the only drugs on which clinical information was available. These drugs include amphotericin B, antimony compounds, ketoconazole, and dapsons. Dapsone has had most attention with detailed descriptions of the inflammatory and healing responses of the host and the effects on the pathogen.

Amphotericin B

Kutty and Teh¹² found amphotericin B to have caused arrest of the development of the pathogen, preventing the recurrence of disease during a three-year follow-up period, and ultrastructural damage to *R. seeberi* was marked. Topical amphotericin B on corneal and nasal rhinosporidiosis have been found to be successful¹³, however, Ho and Tay¹⁴ found the intravenous drug to be ineffective in the treatment of disseminated rhinosporidiosis.

Antimony compounds

Allen and Dave¹⁵ used the antimony compound "Neostibosan" in 18 patients with nasal rhinosporidiosis, with a satisfactory outcome in only three patients. In another case report, there was no recurrence noted after one year after Neostibosan therapy and surgery on nasal rhinosporidiosis.¹⁶ In some in vitro studies¹, the pentavalent antimony compound, sodium stibogluconate, had the highest mean IC₅₀, (55.7 µg/mL) from seven studies, compared with 29.7 µg/mL from 10 studies with dapsons.

Dapsone

Nair¹⁷ noted a significant reduction of recurrence rates from 93 percent to 39 percent in dapsons-untreated and -treated patients during three years, respectively. Job and colleagues¹⁸ concluded that medical therapy alone could replace surgery. The usual

oral dose used was 100 mg/day for durations from 6 months to several years.

Ketoconazole

Only one report exists on the use of ketoconazole. Kunelskaia and colleagues¹⁹ found systemic ketoconazole, topical clotrimazole, and surgery effective in the treatment of nasal rhinosporidiosis.

Neither timethoprim-sulphadiazine nor sodium stibogluconate (or its commercial preparation pentostam) have apparently been used clinically in rhinosporidiosis.

Drugs that were not effective in the MTT-reduction test in vitro included penicillin G, streptomycin, gentamicin, ciprofloxacin, metronidazole, pentamidine, pyrazinamide, isoniazid, and rifampin. Rajam and colleagues²⁰ and Satyanarayana³ found pentamidine ineffective in the therapy of rhinosporidiosis. Other drugs found to be ineffective in vitro¹ have not been used in the therapy of rhinosporidiosis.

Drugs for veterinary use

Two drugs tested by the MTT-reduction method were berenil (diminazine acetate) and imizol (imidocarb dipropionate). Both drugs were found to be more effective with IC₅₀ values (with number of trails in parenthesis) of 13 (5) and 9 (1) µg/mL, respectively than the drugs used mainly in human treatment.¹ There is a limited experimentation with Imizol because the import was stopped.

In vivo tests for drug susceptibility

Mycobacterium leprae, like *R. seeberi*, is uncultivable in vitro, but limited multiplication occurs in the foot-pad of the mice. This model has been used to investigate the susceptibility of *M. leprae* to dapsons.²¹ *R. seeberi* while also being uncultivable in vitro is, however, unable to induce rhinosporidiosis in the foot-

pad of the mice, even though this site has been used to investigate the immune responses to *R. seeberi*, especially cell-mediated immune responses of mice to intra-foot-pad injections of viable rhinosporidial endospores.

The time-course of inhibitory effects of dapsone on *R. seeberi* in vivo and in vitro

Job and colleagues¹⁸ studied the temporal sequences of clinical and histological responses in humans on dapsone therapy for rhinosporidiosis without surgery. A reduction in the lesion size was noted after six weeks, a marked reduction at 36 weeks, and a total disappearance after one year. Mahakrishnan and colleagues recorded a complete regression under dapsone therapy in disseminated rhinosporidiosis within 18 weeks.²² Histologically, the accentuated granulomatous responses and the arrest of the maturation and degeneration or absence of endospores were noted. Venkateswaran and colleagues²³ found no effect on the pathogen until 6 weeks after dapsone therapy, and they noted a reduction in the endospore number, and degeneration or absence of endospores after 36 weeks of therapy. Augmented host responses were noted from the twelfth week of therapy.²⁴ The time-course determinations of the inactivation of rhinosporidial endospores by direct exposure to dapsone in vitro, however, showed that the inactivation commenced on the second day while it was complete on the eighth day (Arseculeratne, 2008, unpublished data). The marked temporal difference between in vivo and in vitro responses is attributable to the time taken for absorption, tissue distribution, and accessibility of orally administered dapsone to the pathogen in the granulomatous rhinosporidial lesions which are surrounded by the barriers of edema, hemorrhage, cell infiltration, cystic spaces, fibrosis, and especially by down-growths of squamous epithelia that surround the

sporangia. Impermeability of sporangia to drugs, postulated by Woodard and Hudson²⁵ as being the cause of failure of drug therapy, has been discounted.¹

Clinical applications

The applicability of anti-rhinosporidial therapy using medication can be considered in two scenarios (a) presurgical or postsurgical and (b) solely medications.

Presurgical use

These comments relate only to dapsone since detailed studies on host and pathogen responses to the other drugs shown to be effective in vitro, are not yet available.

A serious complication of surgery in rhinosporidiosis especially of the nasal and nasopharyngeal sites, is the profuse intraoperative hemorrhage that results from the high vascularity of the growths.²⁶ The responses of patients after medical therapy with dapsone without surgery¹⁸ indicate that the disease process is arrested with the increased resolution and fibrosis. It could therefore be expected that presurgical dapsone would minimize both the hemorrhage by promoting resolution of the infection, with promotion of fibrosis, as well as preventing the colonization and infection of new sites after the release of endospores from the surgically-traumatized polyps. Karunaratne²⁷ has claimed that satellite rhinosporidial polyps result after surgery from the 'autoinoculation' of the endospores, that contaminate the adjacent mucosa during surgery. Presurgical use can be compared with the 'neoadjuvant' chemotherapy of malignant neoplastic disease prior to surgery.

Postsurgical use

Colonization of normal mucosae by the endospores released from the site of excision, could conceivably be controlled by postoperative dapsone. Postoperative use of dapsone has been the commoner mode of treatment.

With dapsone therapy, recurrences have been reported to have been minimized or prevented.¹⁷

In view of the danger of dissemination of *R. seeberi*, especially after surgery, with extensive histolysis of soft tissues including bone and cartilage, it can be considered advisable to commence medications, however, small the original lesion appears to be. Medications can be started even before surgery. The prevention of the spread of overt ocular rhinosporidiosis to the contralateral eye by dapsone has been recorded.²⁸ This emphasizes the urgency of utilizing medications even in the absence of surgery; as ocular rhinosporidiosis with staphyloma formation on the sclera could result in the dangerous complication of scleral perforation and rupture.

Resistance of *R. seeberi* to antimicrobial drugs

There are no data on antimicrobial drug resistance in *R. seeberi*. The strains obtained from human and animal rhinosporidiosis have shown genetic variations, and the possibility exists also of variation of drug susceptibility based on genotype and which might explain the variation of clinical responses to some drugs including amphotericin B and antimony compounds that have been noted in the literature. With mycobacterial infections, notably tuberculosis, a development of resistance to a single drug, as in the early days of prolonged anti-tuberculosis chemotherapy, prompted the use of multiple drugs to forestall development of drug-resistance of *M. tuberculosis*. At present, there are no data on the development of resistance in *R. seeberi* to dapsone. As dapsone is the single most common drug used in the chemotherapy of rhinosporidiosis, it might be prudent to use combinations of several medications. This could be dapsone combined with one or more of the drugs mentioned above, that have been recently shown¹ to have in vitro anti-rhinosporidial activity. Cycloserine which is administered for several

months in anti-tuberculous chemotherapy is, from recent in vitro studies (Arseculeratne, 2008 unpublished data), an effective (IC₅₀ of approximately 10 µg/mL) additional candidate for the multidrug chemotherapy of rhinosporidiosis. Whether cycloserine is synergistic with dapsone is yet to be determined.

The generation time of *R. seeberi* has not been determined as it cannot be cultured in vitro, but this pathogen probably has a long generation time. This is a major justification, as in tuberculosis, for prolonged multidrug combination chemotherapy.

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