LeishRisk: break-out session, 14th Nov 07 Antwerp

"Drug resistance and drug combinations"

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The objectives of the session were outlined (AB), with the central questions being :

1. What effects of drugs should we look for ?
2. What drugs are available ?
3. What types of drugs make useful combinations ?
4. What other supporting work is required ?

(1) What effects of drugs should we look for ?

Speed of parasite clearance:

Considerations:

How important is the speed of parasite clearance (in relation to total load) and do we have the tools to measure this?

Yes, this is an essential parameter to be determined: it demonstrates the efficiency of the drug. The **speed of onset of relapse** may indicate efficiency of drug and/or appearance of resistance. The **rate of relapse** gives overall cure rate at fixed point of time.

Yes, we do have tools to measure the load and weight, but not all ideal

- Splenic aspirates
- Real time PCR, QT-NASBA

Comments and discussion:

There are data on *L. infantum* using RT- PCR (Antinori S. et al., 2007; Cruz I et al 2006; Maurya R et al 2005; Rolao N. et al 2004; Mary, C. et al., 2006) and QT-

NASBA (de Vries PJ, et al., 2006) to monitor parasites in peripheral blood The question is, how accurate is this as an indicator of splenic load ?
Similar studies have been completed in dogs (Oliva G et al., 2006) and tracked blood in relation to bone marrow infection (LG, unpublished data)
What are the limits of sensitivity of RT-PCR in comparison to serology and culture techniques ?
Breakpoints and thresholds need to validated. One suggestion was 1 parasite/ml as the threshold, below that level patients are considered to be asymptomatic (PB) (see Mary,C., et al., 2006).

Other thoughts:
The tools and methodologies developed will be essential in future studies on VL/HIV co-infection cases. At present, we need to have realistic approaches to measure relapse or recrudescence of infection.

Action Points:

- *A more thorough review of the literature is required*
- *Further experimental studies are required to check sensitivity of each technique (GSP with quality control)*
- *Selected methodologies then need to validated in clinical studies*

(2)<u>What markers of drug resistance do we have available ?</u>

<u>Considerations:</u>
At present there are no molecular markers for resistance to any of the standard anti-leishmanial drugs. Of greater concern, there do not appear to be any on the horizon. Specific enzymes have been shown to characterise resistance to miltefosine and amphotericin B in experimental studies, but the relevance to clinical studies is not known.

At present we only determine phenotypic drug resistance in whole cell assays (amastigote/macrophage model) to determine IC50 values. This is not a field adapted assay and requires an established laboratory with trained staff. Central laboratories could be established on a regional basis.

Action points

- *It was noted that a proposal has been submitted to FP7 on surveillance and monitoring of miltefosine and antimonial resistance (SD). This should be followed up when FP7 decisions are known.*
- *Systems also need to be established for monitoring and surveillance of CL though this will be more complex (PB, SC)*
- *There needs to be specific funding to define molecular markers and clinical relevance for amphotericin B, miltefosine and paromomycin.*

(2) What drugs do we have, and (3) what type of combinations would be most appropriate ?

Options for types of drugs to be used in combinations:
The possible combinations were presented (AB) as:
(a) Sequential treatment with a "big hitter" followed by a "mopper upper"[similar to some of the artemisinin combinations used for malaria or rifampicin and dapsone for leprosy. AmBisome + miltefosine is a good example for leishmaniasis]
(b) Synergistic drugs [only Sb and paromomycin in vitro so far reported for *Leishmania*]
(c) Drug plus resistance reversal agent [see current experimental studies on Sb resistance]

Only three options are available for India (given that primary Sb resistance in almost entirely confined to Bihar) are: amp B + milt, ampB + paromo, milt + paromo. Preclinical toxicology (28 day rat study) data on these combinations is available via Dr Rob Don at DNDi (www.dndi.org).

Concerns were raised about:
(a) the AmBisome / miltefosine combination where the long miltefosine tail is "unprotected"
(b) the absence of human pharmacokinetic data to guide the dosing regimes [see work on malaria for comparison, White, NJ, 2002) (PB)
(c) need to adjust combination dosage for children
(d) the need for population PKs for AmBisome and miltefosine (available for paromomycin, see Sundar et al., 2007).
(e) the need to monitor for HIV in trial volunteers and to assess the emergence of resistance in HIV co-infected patients independently.

Other non-front line drugs were also discussed:
(i) allopurinol - used in combination with Sb in limited clinical study in 1980s), is used with Sb in canine leishmaniasis to prevent relapse, in a 1 year therapy. Though successful in unresponsive human VL in Kenya, it was not effective in a small series of unresponsive European patients (AB person comm.). Allopurinol has also been used with pentamidine in reduced dosage in India in one clinical trial in India (Das, VN et al. 2001). Pentamidine is not favoured because of its toxicity. Allopurinol has been used with ketoconazole in a single case of VL (Colakoglu M et al 2006)

(4) What other supporting work is required ?

Models for relapse:
The main additional concern was the choice of a model to study relapse, especially in relation to changes in parasite drug sensitivity during treatment. The dog model was discussed favourably, especially as a study on the treatment of dogs with miltefosine (same dosage as in humans, formulation from Virbac, Fr) is underway in Italy (LG, unpublished). 40 dogs will be monitored and parasite isolates taken before and during treatment. This should enable any change in drug sensitivity to be monitored.
The hamster provides another model of *L. donovani* infection (quantifiable and not spontaneously healing) that has been used successfully for study of development of drug resistance.

Action points

- *Need to reconsider dog model after the canine study of LG is completed*
- *As miltefosine is now being introduced in the Mediterranean region for the treatment of canine leishmaniasis, other studies need to be initiated to monitor drug sensitivity both in relation to the efficacy of the treatment in dogs as well as the selection of a reservoir of resistant parasites that could be transmitted to humans. The centre in Madrid should be contacted via Dr Jorge Alvar.*

Building a process for the collection and archiving of parasite samples:
The need for parasite samples from different regions, different species, from both before and after the introduction of new therapies was considered essential for both research and public health issues. This would provide a strong basis for a monitoring and surveillance system. Four elements were suggested to enable such a system to be established, with suitable funding:

(a) a central bank with full cryo-preservation and management
(b) standard operating procedures for isolation, culture and maintenance
(c) geno-typing according to agreed protocols
(d) phenotypic-drug sensitivity typing according to agreed protocols

Action Points

- *Identification of a centre for the bank, for example Montpellier (contact JP Dedet or Patrick Bastien)*
- *Saskia Decuypere and Graham Coombs to outline a proposal*

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