GLASGOW AND WEST OF SCOTLAND BLOOD TRANSFUSION SERVICE

FUTURE TREATMENT FOR HAEMOPHILIA AND A RECOMBINANT FACTOR VIII

Report of Meeting held at St Thomas' Hospital on 9 May 1990

Milt Mozen: Cutter R & D

New Technologies in FVIII manufacture

Koate HP (plasma derived)

Recombinant VIII

↓ virus, ↑ act/mg, ↑ act/ml, ↓ impurity, improved solubility time enhances presentation.

1984 Koate HT
1985 Use of screened plasma
1986 Koate HS, ALT screen
1987 Monoclonal purified
1989 Koate HP (high purity)

? Recombinant VIII to be licenced

Making HP

Cryo + Heparin Al(OH), PEG, FVIII dissolves

Glycine precipitate

TNBP + Tween Gel filtration Biogel A-5M

TNBP + Tween well removed.

McAb 2 types

Armour Baxter
Cryo Cryo

Anti-vWF TNBP TRITON

Amino hexyl sepharose Anti FVIII c

HT QAE Chromatography

"Monoclone"

Baxter FVIII

Hemophill M

Def'n Specific activity excludes albumin as it's up to maker how much goes in!

T 1/2 assay on Koate HP wanted hi dose option so did experiments on T 1/2 with 2 levels.

T 1/2 and recoveries both OK
Genetic engineered: Into BHK 21
3
26 Exons. Gene is 1/10 of X chromosome.

Uses also part of HBSAg (non-translated) and DHFR (Dihydrofolate
reductase) this is to give selection for high copy numbers using
MTX poisoning! Also put in a neomycin resistance gene.

Adapt to suspension culture clone called R3

After glycosylation MW is 300Kd

N-
Domains are A1, A2, B, A3, C, C' -C
Culture - purify chromatography then McAb purify.

Validation studies - had to compare with very high purity plasma
VIII.

Specific activity depends enormously on methods used (100% error) - probably because total protein is so low.

Immunogenicity

Recombinant FVIII → Rabbit → serum over plasma FVIII column -
test unbound for "neo epitope Ab"

Used a peptide without domain B 90-142-80 (why the numbers?) this
does have an epitope not present on native.

2 years clinical use and is OK

Q: Will the repeated exposure to ng doses of mouse IgG not
ultimately provoke Ab?

A: No evidence. Many tests. Perhaps if inhibitor case
could get a few μcg/d of Ig.

Q: Place of vWF protein co transfection

A: Baxter have this into enhance expression - did he say it
was fractionated out afterwards? Cutter get
expression without it.

Pharmacokinetics of Recombinant FVIII  Harrison

Models include single exponential, double exponential. If you
take many samples can get very complex pattern - multiplicity of
equations added.

The model independent system may not be truly so but gives
recovery, mean residence time and other novel but useful
measures.

Problems with recovery: Test often at 10 minute but peak may be
120 minute by which time clearance is under way!
Choose case

Severe HbPhil A. Small bleed no inhibitor. No Rx for 3d, check dose by assay. Dose >20 iu/Kg and take lots of samples.

Dr Salamantes Mt Sinai, New York (Professor Aledort's paper)

Safety and efficacy of Recombinant FVIII

"Our patients are anecdotally to this point completely thrilled with product"

4 Phase study

(1) Recovery T 1/2
   Safety
   Hi dose prophylaxis (20-40iu/Kg x 3/week)

(2) Home Rx also recovery + T 1/2

(3) Use in patients needing massive doses (surgery etc)

(4) FUPS inc. one Jehovah.

Effective

79.9% responses to single pulse
12.9% needed 2 pulses

Mean annual usage/patient equals approximately other depts with plasma derived FVIII.

The mega dose trial gave one disaster case 2260 iu/Kg in 7 days!

The Jehovah was not a real FUP. Previous Rx on court order. Got hepatitis and inhibitor. Was tolerised.

17 FUPS gave 3 inhibitor cases of Monoclate 8 of 68. Non-sig worse than plasma derived monoclate.

831 infusions 14 ADRs

Chest twinge cold feet 1
Local erythema drip site 2
Pain drip site 1
Strange taste 4
Dizzy 1
Hypotension 2
Mouth dry 1
Rash 2
Muscle tightness ?
Any exposure to human derived coagulants is perceived as unacceptable by these patients who are delighted with product.

Discussion

Kernoff - what do patients want and should they be given it.

Bloom - The incidence of inhibitor in PUPs is important. Previously treated patients without inhibitor might be selected therefore not going to make inhibitors.

Q: Transaminitis
A: (Kernoff) case seen approximately three monthly but when recombinant FVIII given far more blood is taken. Cannot attribute it to product. There were pre-existing abnormalities. Salamantes - no ALT elevation in PUPs others already had upsets which continues.

Colvin: How many patient years study to find one single advantage over best plasma products?
A: Laughter

Wensley: If light chain > heavy chain why, what does it mean?
A: Mozen: Assay dependent. No one knows which is right. Some XS of 80Kd? Because of epitope used by monoclonal purification method.

Performance post infusion could be explained by a more rapid excretion of an inactive peptide fragment.

Q: Cost?
A: Don't know

Q: Production capacity
A: As much as market will buy

Comment: But that is wholly dependent on price!

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21.05.90

D: MT-HAEM