DIHYDROSTREPTOMYCIN/STREPTOMYCIN

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ADDENDUM
to the dihydrostreptomycin/streptomycin monograph
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Streptomycin and dihydrostreptomycin were evaluated by the Committee at its 43rd meeting in November 1994. The Committee proposed a temporary ADI for streptomycin and dihydrostreptomycin. Temporary MRL's of 500 µg/kg (muscle, liver, fat), 1000 µg/kg (kidney) and 200 µg/l (milk) were also recommended, expressed as the sum of streptomycin and dihydrostreptomycin. Insufficient data was available to support an MRL for eggs.

The following information on residues was requested by the Committee for evaluation in 1997.

1. An evaluation report or results of experimental studies on the metabolism of dihydrostreptomycin and streptomycin.

2. Data on residues of streptomycin and dihydrostreptomycin in eggs.

3. Results of studies to determine the relationship between the antimicrobial activity of the residues and their concentration, as measured by specific chemical methods.

The purpose of this evaluation is to address these points.

Metabolism

The Committee at its 43rd meeting stated that "very little information is available on the metabolism of either streptomycin or dihydrostreptomycin in farm animals; the drugs are probably not extensively metabolised". No subsequent experimental studies have been carried out to determine the metabolism of streptomycin and dihydrostreptomycin. Biotransformation studies, using radiolabelled streptomycin or dihydrostreptomycin in laboratory animals, farm animals and possibly man using in vitro techniques (e.g. exposure of labeled streptomycin or dihydrostreptomycin to gut flora) would involve extensive study. The sponsors suggest that biotransformation data on aminoglycosides are limited because of difficulties in obtaining radiolabeled material of sufficient purity and the lack of applicable separation methodologies and effective spectroscopic techniques. They also argue that if the parent compounds are little metabolised then only minor metabolites would be expected and it is difficult to see how knowledge of the identity of such metabolites would contribute to a more informative safety assessment.

The sponsors have submitted an evaluation report on the metabolism of both drugs (Norbrook, 1996). Background pharmacokinetic data (absorption, distribution, excretion) on streptomycin and dihydrostreptomycin are available for the oral and parenteral routes of administration in laboratory and farm animals and man. Literature searches (Medline 1966-1996; Embase 1974-1996), however, have hitherto failed to reveal any published data on the metabolism (biotransformation) of streptomycin or dihydrostreptomycin.

Aminoglycosides as a class are reported not to be metabolised in humans (Pratt & Fekaty, 1986). Because of their high molecular weight unmetabolised streptomycin or dihydrostreptomycin are unlikely to be absorbed from the gut following oral administration, e.g. 60-100% of an oral dose of streptomycin was excreted unchanged in the faeces of man (Dollery, 1991). Also if the aminoglycosides are not readily metabolised then the drugs should be excreted unchanged in the urine following parenteral administration, e.g. after parenteral administration of streptomycin 50-60% of a dose is excreted unchanged in the urine within 24 hours in man (Anderson & Jewell, 1945). No data or references on the biotransformation of dihydrostreptomycin were provided.

Two other aminoglycosides, gentamicin and neomycin, were evaluated at the 43rd meeting of the Committee and evidence was given demonstrating that following radiolabeled studies these aminoglycosides were not metabolised in

farm animals. Although various reviews make claims about the lack of biotransformation of aminoglycosides as a class following oral or parenteral administration, it is difficult to locate hard data on which most of these statements are based. One study was that using ^{14}C-neomycin in calves by Aschbacher & Feil (1994). Nevertheless, even in this study, unchanged parent compound was found present in kidney tissue and faeces, but no metabolites were identified in tissues or excreta. If biotransformation were to take place, one would expect metabolites to be formed mainly in the liver or kidney following parenteral administration and mainly in the intestine following oral administration.

Although there is no direct evidence showing whether the parent aminoglycoside or a metabolite is responsible for the two main forms ototoxicity (nephrotoxicity and ototoxicity), mechanistic studies on nephrotoxicity would indicate involvement by a reactive oxygen species induced by the parent aminoglycoside rather than a direct action of a reactive metabolite of the aminoglycoside on the target organ (see review of Forge & Harpur, 1993).

Residues in Eggs

The sponsor does not have or intend to apply for a license to use the drugs in laying birds. Thus they have not submitted further information on residues in eggs. Nevertheless, the drugs are in preparations from other companies and could be used in laying birds.

Analytical Methods

The sponsors have submitted an HPLC method for dihydrostreptomycin (but not streptomycin) and determined the relationship between the antimicrobial activity of residues of dihydrostreptomycin and the concentration of the drug in fortified ovine liver and kidney tissues. The specific chemical (HPLC) assay for dihydrostreptomycin has an LOQ of 400 µg/kg (Norbrook, SOM No. CRD/DST/010).

Fortified ovine liver and kidney tissue samples (10 g) were prepared at two concentrations of dihydrostreptomycin (500 µg/kg and 1200 µg/kg) and assayed in parallel by both the HPLC assay and Norbrook's standard in-house microbiological assay (Norbrook, SOM No.MRD/DST/0l0) which has an LOQ of 400 µg/kg. The results are shown in Table 1.

Table 1. Comparison of results (µg/kg) for the analysis of dihydrostreptomycin by an HPLC and a bioassay method

Fortification (µg/kg)	Sheep Liver		Sheep Kidney	
	HPLC	Bioassay	HPLC	Bioassay
500	491 - 510	474 - 501	491 - 500	477 - 489
1200	1150 - 1280	1170 - 1190	1140 - 1220	1130 - 1170

The results of this experiment confirm that the results for fortified samples obtained, if based on antimicrobial activity, are equivalent to results obtained from a specific chemical assay.

The sponsors have developed an HPLC method for the measurement of dihydrostreptomycin in muscle, liver, kidney and fat of cattle, sheep and pigs. The tissue was deproteinised with perchloric acid and the liquid extract was placed onto a small solid phase column, washed with water and the dihydrostreptomycin eluted with phosphate buffer, pH 7.5. The eluate was analysed by HPLC with post-column derivatisation and fluorescent detection.

The in-house validation was carried out using fortified tissues in the range 400 - 5000 µg/kg tissue. The accuracy of the method was measured by using replicates at 400 and 5000 µg/kg spikes. The LOQ was determined as 400 µg/kg for all tissues.

A summary of the in-house validation results is shown in Table 2.

Table 2. **Assay criteria for the HPLC method for dihydrostreptomycin in edible tissues of cattle, sheep and pigs**

Species/ Tissue	r^2 for range 400 -5000 µg/kg	Accuracy (%) and (CV) (%)	
		Fortification 400 µg/kg	Fortification 5000 µg/kg
Cattle			
Muscle	0.997	89 (3.1)	88 (3.3)
Liver	0.991	103 (0)	106 (1.5)
Kidney	0.996	87 (10.6)	87 (3.2)
Fat	0.997	98 (2.6)	99 (1.6)
Sheep			
Muscle	0.995 (day 1) 0.990 (day 2)	109 (3.7) 97 (3.9)	NM 91 (5.4)
Liver	0.995	106 (3.1)	92 (1.3)
Kidney	0.992	107 (2.5)	91 (2.0)
Fat	0.995	102 (0)	89 (1.4)
Pig			
Muscle	0.993	102 (0)	95 (0.6)
Liver	0.997	105 (0)	106 (0.8)
Kidney	0.996	101 (3.7)	88 (4.3)
Fat/skin	0.995	105 (3.1)	97 (1.3)

NM = Not measured

The sponsors have carried out tissue residue studies in cattle, sheep and pigs for their penicillin /dihydrostreptomycin combination product, (PEN & STREP) using this HPLC method for dihydrostreptomycin. The results are given in Table 3 together with some results using the bioassay (microbiological) method.

Table 3. **Residues of dihydrostreptomycin (μg/kg) in tissues of cattle, sheep and pigs after intramuscular injection with a procaine penicillin/dihydrostreptomycin preparation. The dose of dihydrostreptomycin sulphate was 10 mg/kg body weight once daily for three days**

Species/withdrawal time (days)	Number of animals	Muscle, liver, kidney and fat		Injection site HPLC (μg/kg)
		HPLC (μg/kg)	Bioassay (μg/kg)	
Cattle 14	4	<400		982, 954, <400, <400
18	4	<400	<1000	1140, <400, <400, <400
21	4	<400		<400
28	4		<100	
Sheep 14	4	<400		982, 455, 681, 417
18	4	<400	<1000	691, 621, 440, <400
28	4	<400	<100	<400
Pigs 14	4	<400		<400
18	4	<400	<1000	<400
28	4		<100	

HPLC LOQ = 400 μg/kg. In the bioassay the LOD was either 1000 or 100 μg/kg.

The results show that with this one preparation containing procaine penicillin and dihydrostreptomycin no residues >LOQ were found in the edible tissues except at the injection site. At the withdrawal times of 18 days or longer no residues were found by the bioassay method when similar experiments were carried out by this sponsor.

APPRAISAL

No new studies have been carried out on the metabolism of either streptomycin or dihydrostreptomycin. An expert report was provided, however, that inducted:

1. Background pharmacokinetic information on the oral route of administration to animals and humans that suggest there is little metabolism of either drug.

2. Aminoglycosides as a class of antimicrobials are not readily metabolized in humans. In particular studies using radio-labeled neomycin in cattle, no metabolites other than parent drug were identified in tissues or excreta.

3 Both streptomycin and dihydrostreptomycin are unlikely to be absorbed from the gastro-intestinal tract because of their high molecular weight, and they are excreted unchanged in faeces.

4. When administered parenterally to humans, most of the streptomycin was excreted unchanged in the urine.

Based on the expert report the Committee recommended that further information on metabolism is not essential for the following reasons:

1. Extrapolation from limited studies with other aminoglycosides in farm animals supports a strong indication that both streptomycin and dihydrostreptomycin remain unmetabolised in farm animals and humans.

2. Additional studies may not yield substantial new information.

The sponsor has not submitted further information on residues in eggs because it does not have or intend to use the drugs in laying birds. Therefore, the Committee is not in a position to recommend an MRL for eggs.

An HPLC method was developed for the measurement of dihydrostreptomycin in muscle, liver, kidney and fat of cattle, sheep and pigs. The extracts were analysed by HPLC with post-column derivatisation and fluorescent detection. The method evaluation was carried out using fortified tissues with concentrations of 400 - 5000 µg/kg tissue. The method was linear over this range and the accuracy of the method was acceptable. The limit of quantification (LOQ) was determined as 400 µg/kg for all tissues. There was no interference from streptomycin but the specificity towards other aminoglycosides and antibiotics was not determined.

The HPLC method and the antimicrobial assay were compared using sheep liver and kidney tissues fortified with dihydrostreptomycin at 500 µg/kg and 1200 µg/kg. The results for the two methods were equivalent. No comparison of the methods was made using incurred tissues.

In treated animals residues were below the LOQ (400 µg/kg) in the edible tissues of cattle, sheep and pigs at 14 days or thereafter following daily injections over a three day period at the recommended dosing (10 mg dihydrostreptomycin sulphate/kg body weight) of a preparation of dihydrostreptomycin combined with procaine penicillin. Similar results were observed in earlier studies when an antimicrobial assay was used. Residues were found in tissues collected from the injection site of cattle and sheep at 14 and 18 days after dosing but were below the LOQ at 21 and 28 days post dosing, respectively. Residues at the injection site in cattle and sheep showed broad variability at 14 and 18 days following treatment. At 14 days post treatment residues in cattle were <400-982 µg/kg and at 18 days, <400-1140 µg/kg. In sheep, at 14 days residues were 417-982 µg/kg and at 18 days, <400-691 µg/kg. No residues were found at the injection sites in pigs sacrificed at 14 and 18 days after dosing.

Conclusions:

1. Additional information on the metabolism of dihydrostreptomycin and streptomycin is not required.

2. No MRLs can be recommended for eggs because additional data was not made available to the Committee for residues in eggs.

3. The HPLC analytical method is satisfactory for monitoring for residues of dihydrostreptomycin but has not been tested for streptomycin. Therefore, the method can not be used to regulate the MRL which is set as the sum of the two drugs.

   The Committee affirmed maintaining the temporary MRLs recommended by its 43rd meeting.

   The sponsor was asked to supply the following information for evaluation in 1999:

   -Validation of the HPLC assay to measure residues of streptomycin.

   -Studies to determine whether the HPLC and bioassay methods give similar results for residues of both drugs using tissues with incurrent residues.

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