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ASSESSMENT OF THE RESIDUES IN RABBIT AFTER ORAL TREATMENT WITH AMINOSIDINE SULPHATE IN WATER.

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The pharmacokinetics of the aminosidine sulphate administered by oral route has been studied in cattle, sheep, pigs and dogs (Pignattelli and Faustini, 1967) and in calves (Girardi et all. 1986).
The addition of aminosidine sulphate in the rabbit's diet turned out to be very useful for the prevention of enteric syndromes (Tocchini and Tardani, 1975).
Recently it has been shown a high tolerability of the aminosidine sulphate in rabbits after weaning and a slight entero-absorbtion (Cringoli et all. 1988).
Also recently have been reviewed the lethal effects of some antibiotics which may be observed in rabbits. (Lumeij at all. 1987).
In this work, after the administration of aminosidine sulphate by oral route in weaned rabbits for 7 consecutive days, it has been determined the quantity of antibiotic excreted with faeces. Also the concentrations in blood, kidney, muscle, lung, liver, heart, spleen and the intestinal content has been determinated.

MATERIALS AND METHODS

The trials have been carried out in 12 weaned New Zeland young femal rabbits of an average weight of 550 gr.

The rabbits belonged to the same breeding farm.
The animals were put in single cages, with a device to collect the faeces excreted during trial period. The animals were fed with feed in pellets ad libitum and watered with feeding bottles of 500 ml capacity.
The feed used did not contain neither antibiotics nor inhibent substances and it has been especially prepared for the trial.
Before starting the trial, the rabbits were adapted to the new environment for 3 days and the faeces were checked for the presence of possible inhibent substances.
Out of 12 rabbits, 9 were used for the treatment, while the other 3 were used as control.
Each rabbit was administered, by oral route, a daily dose of 80 mg of aminosidine sulphate in solution.
The treatment was repeated for 7 consecutive days.
Each rabbit was easily administered the dose of aminosidine, in an unique volume of 0.5 ml, by means of an insulin syringe (without needle) directly in os.
Every morning, till the sacrification of all animals, both the control animal's and treated animal's faeces have been collected and weighted.
We utilised this quantity of faeces to determine the aminosidine excreted.
Afterwards there was the preparation of a pool of faeces by withdrawing the 5% of each treated animal's sample.
The daily faeces quantity of each animal fluctuated around 140 gr.
At the end of the treatment the animals were sacrificed as follows:
- 3 treated rabbits and 1 control were sacrificed 2 h. after the last treatment (2 h.).
- 3 treated rabbits and 1 control were sacrificed 48 h. after the last treatment (48 h.)
- 3 treated rabbits and 1 control were sacrificed 7 days after the last

treatment (7 days)

From each rabbit was collected blood, kidney, muscle, lung, liver, heart, spleen and intestinal content (the content of colon and caecum which was weighted).

The determination of levels and residues of aminosidine has been executed using the microbiologic tests in agar germ holes using the Bacillus subtilis, strain ATCC 6633 (CEE reports, 1985).

To fit the standards curves to the serum of the control rabbit 0.1 ml aminosidine sulphate in phosphates buffer at pH 8.0 was added to obtain an antibiotic concentration of 1.25 mg/ml of serum.

Other 3 dilutions were prepared by doubling from this solution, with phosphates buffer at pH 8.0.

Extraction from organs and tissues.

Each organ, from control rabbit, is separately added to a tricloroacetic acid solution at 10% in proportion of 1 gr. of organ or tissue and 2 ml of solution.

Then they were homogenized in ultraturrax for a few minutes.

The obtained homogenate is centrifuged at 8000 rotations per minute (r. p. m.) for 10 minutes.

The floating liquid, is neutralised at pH 7.0 with a solution of 20% NaOH.

An aminosidine sulphate solution is added to the prepared extracts to obtain a concentration of 2.5 mcg/ml of extract.

4 doses of the standard (mcg/ml 1.25 - 0.62 - 0.31 - 0.16) are prepared from this solution, after careful agitation, diluiting by doubling with phosphates buffer solution.

As for faeces and intestinal content of the control rabbits aminosidine sulphate was added to obtain the 10 - 5 - 2.5 - 1.25 mcg/ml concentrations to the fitting of standard curves.

Extraction from tissues and organs of rabbits treated with aminosidine

sulphate.

A double volume of a tricloroacetic acid solution at 10% has been added to 1.0 - 1.5 gr. of organ or tissue.

After a few minutes of homogenizing in ultraturrax the homogenate has been centrifuged at 8000 rounds r. p. m. for 10 minutes.

The floating is collected and neutralised with a NaOH solution at 20% and then utilised or further diluted for the research of the aminosidine's residues.

Faeces and intestinal contents of rabbits treated with aminosidine sulphate, after weighting, have been titrated either singularly or in pool, taking up a percentage of 5% in weight.

The above mentioned quantity of faeces or intestinal contents have been submitted to extraction and treatment as described for organs and tissues.

When the quantity of residues have consented it, residue determinations were carried out proceeding at further dilutions.

Serums of treated rabbits were utilised such as they are.

RESULTS

Either rabbits, treated with aminosidine sulphate (A.S.), or control rabbits, have regularly assumed feed during the trial period and have shown a rapid, satisfactory, weight's increase.

At the sacrification of the rabbits after 2 h. from the end of the treatment, the A.S. has been found in poor quantity in blood and kidneys as pointed out in TAB. 1 and in high quantity in the intestinal content TAB. 2.

Into the 3 rabbits sacrificed at 48 h. from the end of treatment, the A.S. has been found only in the serum of one animal at quantity below 0.16 mcg/ml.

Into the 3 rabbits sacrificed 7 days after the end of the treatment no permanence of A.S. has been noticed.
Finally, in TAB. 3, is reported the quantity of A.S. found in the animal's faeces both during and after the treatment.

CONCLUSIONS

The results of this assessment point out:
- A poor entero-absorbtion of A.S., since no traces of A.S., according to the sensitivity of the method (0.16 mcg/ml-gr), were detected in muscle and organs, except in kidneys and serum, still 2 hours after the last dosing.
- The presence of residues disappear soon even in the serum and kidney. Only one rabbit showed a presence of residues in the serum, at the limit of the microbiological assay, 48 h. after the last dosing.
- Aminosidine sulphate is found at high concentrations in the intestinal contents (caecum and colon) of animals sacrificed 2 hours after the last dosing, while it is absent in animals sacrificed 48 h. after.
- A.S. is found in faeces after 24 hours from the first administration. A.S. in faeces is high during the treatment and till after 24 hours, it remarkably decreases after 48 h. and disappears after 72 hours from the end of the treatment.

This assay points out how the entero-absorbtion is poor also in rabbits and the dose supplied and assumpted per os is almost totally, more than 80%, and quickly excreted with faeces.
These results confirm the A.S. is scarcely absorbed at intestinal level and it is well tolerated by rabbits. (Cringoli, 1988).
It has to be noted that A.S. administered at high dosages (150 mg/kg by weight at start) for 7 days leaves no traces in the rabbit's meat still 2 hours after the oral treatment and disappears also in the serum and

kidney within 48 hours.

It should be interesting to go deeply through the study of pharmacokinetics of A.S. administered by oral route in drinking water or in feed to rabbits of different ages.

Therefore, this antibiotic could find a wider application in the animal health field.

TABLE 1 - Aminosidine sulphate (A.S.) residues (mcg/ml - gr) in rabbits sacrificed 2 hours after the daily treatment of 80 mg A.S. administered for 7 days

rabbit n°	serum	kidney	muscle	lung	liver	heart	spleen
1	-	1	-	-	-	-	-
2	0,5	1,8	-	-	-	-	-
3	-	1,2	-	-	-	-	-

The mark - shows absence of any inhibition's trace

TABLE 2 - Aminosidine sulphate (A.S.) recovered in the intestinal content: of rabbits treated orally for 7 days at the dose level of 80 mg and sacrificed 2 hours after the last dosing

rabbit n°	recovered A.S mg/rabbit	average recovered mg/rabbit
1	80,80	
2	97,75	91,4
3	94,87	

TABLE 3 - Aminosidine sulphate (A.S.) recovered in pool of rabbit's faeces, treated per os with 80 mg of antibiotic every 24 hours for 7 consecutive days

Treatment	excreted faeces n°	trial period in hours	aggregate average daily quantity of A.S. expressed in mg, recovered in each rabbit's faeces	
1	1	0*		-
2	2	0-24		43,50
3	3	24-48		74,20
4	4	48-72		67,95
5	5	72-96		55,00
6	6	96-120		57,60
7	7	120-144		73,40
	8	0-24**		74,70
	9	24-48		10,80
	10	48-72		-
			A.S.recovered from each rabbit	457,15
			aggregate dose of A.S. administered	560
			% A.S. recovery	81,63

* Faeces collected just before starting the treatment
** Faeces collected after the end of antibiotic treatment
The mark - shows absence of antibiotic

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Aminosidine sulphate was given by oral route once a day for 7 consecutive days to weaned New Zealand rabbits at the dose level of 80 mg. via drinking water.
The amount of antibiotic excreted with faeces and the presence of residues in tissues and organs of rabbits sacrificed after 2 and 48 hours and 7 days after the last dosing, were measured by microbiogical assay.
More than 95% of aminosidine sulphate was recovered in faeces.
Low concentrations were detected in kidneys and blood of rabbits sacrificed 2 hours after the last dosing.
No traces of aminosidine sulphate were detected in samples of muscle, liver, lung, heart and spleen.
The results show how aminosidine sulphate be very scarcely absorbed through the rabbit gut after weaning.

RICERCA DEI RESIDUI IN CONIGLI TRATTATI PER VIA ORALE, CON AMMINOSIDINA SOLFATO IN ACQUA

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A conigli di razza Nuova Zelanda, dopo lo svezzamento, è stata somministrata per via orale l'amminosidina solfato alla dose di 80 mg ogni 24 ore, per 7 giorni consecutivi.
Con il metodo di titolazione microbiologica, è stata determinata la quantità di antibiotico eliminata attraverso le feci, e la presenza di residui nei tessuti ed organi dei conigli abbattuti dopo 2 ore, 48 ore e 7 giorni dalla fine del trattamento.
E' stato rilevato che l'amminosidina solfato viene eliminata attraverso le feci per oltre il 95%.
Basse concentrazioni di antibiotico sono state rilevate solamente nei reni e nel sangue degli animali abbattuti 2 ore dopo la fine del trattamento.
L'antibiotico è risultato non titolabile od assente nei campioni di muscolo, fegato, polmone, cuore e milza.
I risultati riportati provano lo scarsissimo entero assorbimento dell'amminosidina solfato, allorchè somministrata al coniglio durante il periodo dello svezzamento.

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