DETECTION OF RESIDUES OF CHLORAMPHENICOL IN SLAUGHTERED PIGS.

STEFAN FABIANSON and ÅKE RUTEGÅRD

Dept of Food Hygiene, The Veterinary College, Stockholm, Sweden

Tissue concentrations of chloramphenicol in pigs have been studied up to six days after intramuscular administration of chloramphenicol 20 mg/kg bodyweight.
The tissue concentrations never exceeded the sensitivity level for the official biological method used in Sweden for detecting antibiotic residues in meat. Other biological methods for detecting antibiotic residues were compared. All failed to detect chloramphenicol at current dosage in pigs.

By biological determination of urine samples it was possible to detect chloramphenicol during the first day after an intramuscular injection.

Using gas liquid chromatography chloramphenicol in tissues could be detected up to six days after the injection.

DECOUVERTE DE RESIDUS DE CHLORAMPHENICOL DANS LES PORCS ABATTUS

STEFAN FABIANSON et ÅKE RUTEGÅRD

Division d'Hygiène Alimentaire, Ecole Supérieure de Vétérinaires, 104 05 Stockhlom, Suède

Des concentrations de chloramphénicol ont pu être étudiées dans le tissu de porcs jusqu'à six jours après une administration intramusculaire de chloramphénicol de 20 mg par kilo de poids de l'animal.

Ces concentrations dans le tissu ne dépassaient jamais le niveau de sensibilité du procédé biologique officiel employé en Suède pour découvrir des résidus d'antibiotiques dans la viande. Une comparaison a été faite avec d'autres procédés biologiques pour découvrir des résidus d'antibiotiques. Aucun de ceux-ci ne parvenait à découvrir le chloramphénicol administré à des porcs en doses courantes.

Par une détermination biologique de spécimens d'urine, il a été possible de découvrir du chloramphénicol au cours de premier jour après une injection intramusculaire.

Grâce à l'emploi de la chromatographie en phase gazeuse, le chloramphénicol a pu être découvert dans les tissus jusqu'à six jours après une injection.

F3:2

ENTDECKUNG VON RÜCKSTÄNDEN VON CHLORAMPHENIKOL IN GESCHLACHTETEN SCHWEINEN

STEFAN FABIANSON und ÅKE RUTEGÅRD

Tierärztliche Hochschule, Abteilung für Lebensmittelhygiene, 104 05 Stockholm, Schweden

Chloramphenikolverdichtungen sind bis zu sechs Tagen nach einer intramuskulären Injektion von Chloramphenikol von 20 mg je Kilogramm Körpergewicht in dem Gewebe von Schweinen studiert worden.

Die Verdichtungen in dem Gewebe überschritten nie das Empfindlichkeitsniveau des in Schweden benutzten amtlichen biologischen Verfahrens, um Rückstände von Antibiotica in Fleisch zu entdecken. Andere biologische Verfahren zur Entdeckung von Rückständen von Antibiotica sind verglichen worden. Keines von diesen konnte das in normalen Dosen an Schweinen injizierte Chloramphenikol entdecken.

Durch biologische Determination von Harnproben war es möglich, während des ersten Tages nach einer intramuskulären Injektion Chloramphenikol zu entdecken..

Bei Anwendung der Gas-Chromatographie konnte das Chloramphenikol bis zu sechs Tagen nach einer Injektion in Geweben entdeckt werden.

ОБНАРУЖЕНИЕ ОСТАТКОВ ЛЕВОМИЦЕТИНА В СВИНЬЯХ ПОСЛЕ УБОЯ

Стефан Фабиансон и Оке Рутегорд

Ветеринарный институт, отделение гигиены питания

104 05 Стокгольм, Швеция

РЕЗЮМЕ

Изучалась концентрация левомицетина в тканях свиней на протяжении 6 дней после его внутримышечного введения в дозе 20 мг/кг веса туши.

Концентрация левомицетина в тканях ни разу не превышала предела чувствительности официального биологического метода, применяемого в Швеции для обнаружения остатков антибиотиков в мясе. Сравнивались также другие биологические методы обнаружения остатков антибиотиков. Ни один из них не был в состоянии выявить в тушах свиней обычные дозы левомицетина.

Применительно к пробам мочи биологический метод позволял обнаруживать левомицетин в течение первого дня после внутримышечной инъекции.

С помощью газовой и жидкостной хроматографии левомицетин в тканях удавалось обнаруживать на протяжении 6 дней после инъекции.

DETECTION OF RESIDUES OF CHLORAMPHENICOL IN SLAUGHTERED PIGS

STEFAN FABIANSON and ÅKE RUTEGÅRD

Dept. of Food Hygiene, The Veterinary College, 104 05 Stockholm 50, Sweden

INTRODUCTION

Chloramphenicol is an antibiotic drug with a broad antimicrobial spectrum. It is used in both man and animals. The administration of chloramphenicol to man has, in rare cases, when relatively high doses were used caused serious toxic effects in the form of agranulocytosis and aplastic anemia (Wintrobe, 1961).

According to current literature, chloramphenicol is rapidly excreted in most animal species. Most of the studies are carried out as assays of chloramphenicol concentrations in the serum only, e.g. in the dog (Pauli and English, 1971, Watson, 1972), the horse (English and Withy, 1959, Oh-Ishi, 1968), the pig (English and Seawright, 1961) and cattle (Sisodia et al. 1973, Ziv et al. 1973). In order to establish a safe withdrawal time between administration of chloramphenicol to meat producing animals and slaughter, the excretion pattern in the pig was studied during six days following a single intramuscular injection (Fabianson et al. 1976). During this investigation it was found that when chloramphenicol was administered to pigs at current dosage, it was at any time impossible to detect residues in the tissues by means of the official biological method used in Sweden to detect antibiotic residues in meat. By using gas chromatography it was possible to detect chloramphenicol concentrations as low as 0,01 µg/g of tissue. It was found that the mean concentration of chloramphenicol in the muscles reached a maximum of 3 µg/g three hours after the injection and then showed a fast decrease to 0,03 µg/g three days after the injection. By the official biological method the detection limit was around 5 µg/g, and it therefore was impossible to get a positive reaction with this method. It was also shown that the renal concentration did not exceed the muscle concentration during the excretion phase and for this reason biological examination of kidney tissue could not be used in order to get a margin of safety for the determination of chloramphenicol in pig carcases. In order to further test the biological detection of chloramphenicol residues in slaughtered animals the following investigation was undertaken.

MATERIALS AND METHODS

Experimental animals

A total of 13 pigs of Swedish land race were used. The weight of the pigs at the start of the investigation varied from 20 to 40 kg. The pigs were fed a feed mixture free of any antibiotic or growth stimulating substance.

Administration of chloramphenicol

Chloromycetin®, suspension in water, 150 mg/ml ad us vet, Parke-Davis, was used in all experiments. Eleven animals were given an intramuscular injection of the drug in the neck five cm behind the base of the ear. The dose corresponded to 15 mg/kg body weight for one animal, 20 mg/kg body weight for eight animals, 25 mg/kg body weight for one animal and 35 mg/kg body weight for one animal.

Collection of samples

Six animals which had received chloramphenicol injections of 20 mg/kg were slaughtered 3, 6 and 18 hours after the injection. The animals which had received injections of chloramphenicol of 25 mg/kg and 35 mg/kg were slaughtered one hour after the injection. Two animals served as controls. Samples were taken from kidneys and muscles. All samples were frozen before analysis. Urine samples were collected from three animals (one control) by aspirating urine from the bladder by means of a syringe passed through the abdominal wall. From six animals the urine was collected at slaughter.

Biological determination

The bacterial inhibition from urine - and kidney samples was compared using four different biological methods.

1) The official method for the determination of antibiotic residues in meat given by the Swedish Veterinary Board in Regulation V.F. 1966:10. Strain: Micrococcus luteus ATCC 9341. Medium: Peptone free agar (Oxoid no. CM 349) at pH 7,4. Five ml of peptone free agar was poured into petri dishes. After solidifying and drying, the surface was inoculated with an 18 hour broth culture of M. luteus which had been incubated at 30°C and diluted 1:5 with physiological saline solution. The excess liquid was removed by a pipette and the plate dried for 30 min. in a 37°C incubator. Kidney samples, approximately 15 mm in diameter (the size of a hazelnut) and filter discs (10 mm Ø, Schleiser & Schüll no. 2208) impregnated with 0,08 ml of urine, were placed on the agar surface. The plates were refrigerated at + 4°C for 4 hours to allow the inhibiting substance

present to diffuse into the agar, whereupon the plates were incubated for 18 hours at +30°C.

2) A modification of the official method of the German Federal Republic, 4 Abteilung, AB.A. Strain: Bacillus subtilis BGA (Merck). Medium: Peptone free agar (Oxoid no. CM 349) at pH 7,4. Peptone free agar was melted and cooled to 50°C. One ml of a standardized sporsuspension of B. subtilis BGA (Merck) was added to 100 ml of the melted agar and this medium was poured in 5 ml portions into petri dishes. The samples were prepared and placed on the plates as mentioned above and the plates allowed to stand for 3 hours in room temperature before incubation in 30°C for 18-24 hours.

3) Modification of the previous methods using various bacterial strains.

a) Strain: Bacillus stearothermophilus var. calidolactis C 953. Medium: TDYM-agar (Tryptone 15 g, Meat extract 3 g, Yeast extract 5 g, Peptonized milk powder 15 g, Glucose 1 g, Agar 15 g suspended in 1000 ml dest. water) at pH 7,4.

The TDYM-agar was melted and cooled to 55°C. Two milliliters of an 18 hour broth culture of B. stearothermophilus which had been incubated at 65°C was added to 100 ml of the agar. The inoculated agar was poured in 5 milliliter portions into petri dishes and solidified and the samples were placed on the agar surface. Inhibiting substance present was allowed to diffuse into the agar during 3 hours in room temperature, whereupon the plates were incubated 18 hours at 50°C.

b) Strain: Bacillus subtilis ATCC 6633. Medium: Peptone free agar (Oxoid no. CM 349). Three milliliters of an 18 hour broth culture of B. subtilis, which had been incubated at 37°C was added to 100 ml of melted and cooled agar. The plates were prepared as mentioned above. After standing 3 hours in room temperature the plates were incubated 18 hours at 30°C.

The diameter in millimeter of the inhibition zones around the tissue specimens and discs (measured as the space between the border of the sample and the border of the inhibited area) was recorded.

RESULTS AND DISCUSSION

The results are given in table 1 and 2. No visible zone of inhibition was detected around the kidney specimens in the M. luteus test when a dose of 20 mg chloramphenicol per kg had been administered. The recommended dosage in pigs is around 12 mg/kg bodyweight (Parke-Davis, Sweden). Three other methods for detecting antibiotic residues in meat were compared with the M. luteus test. The B. subtilis BGA and the B. subtilis ATCC 6633 both showed a narrow and unspecific zone of inhibition, while the B. stearothermophilus showed an inhibition zone of 8 millimeters. A similar zone on the B. stearothermophilus plates appeared around kidney specimen of the control animals and must therefore be caused by an unspecific substance. There are previous reports of unspecific inhibition of bacteria in biological examination of kidneys after freezing the samples (Forschner and Glende, 1976).

From the experiments carried out it is obvious that neither of the methods was suitable for detecting chloramphenicol residues after an intramuscular injection at current dosage.

At three times the current dosage (3 x 12 mg/kg) a narrow, but distinct zone of inhibition could be seen around one kidney specimen taken one hour after the injection of chloramphenicol. In one case, when only 15 mg/kg body weight was used, an inhibition zone of 4 millimeters was recorded four hours after the injection (table 1). We have not been able to explain this fact, which is in contrast to all our other findings.

Using urine samples it was possible to detect residues of chloramphenicol 1 to 6 hours, but not 18 hours, after an intramuscular injection. Contrary to when kidney specimens were examined no false controls were recorded when using urine samples.

The largest zones of inhibition were recorded by using B. subtilis ATCC 6633.

According to Grove and Randall (1955) biological determination of chloramphenicol is not suitable because of the relation between the level of sensitivity of the method and the serum and tissue concentrations which are obtained at current dosage levels. These tissue concentrations seem to be too low to be harmful to the consumer of the meat, particularly since chloramphenicol does not cause allergic reactions (Schwank and Jirasek, 1963, Lammers et al. 1961). However, the residues of chloramphenicol could present a problem in the bacteriological examination of carcases since the concentration could be sufficiently high to cause inhibition of the outgrowth of bacteria present in the tissues. It is obvious, from the results obtained, that kidney tissue can not be used in a biological control of antibiotic residues to detect earlier chloramphenicol treatment. However, when urine samples are used, it is possible to detect chloramphenicol residues in pigs up to a few hours after the administration of current dosages. After 18 hours the urine samples are also negative. The only method available to detect chloramphenicol after this time is using chemical analysis, and preferably gas chromatography. This is an expensive and time consuming method and useful only for sample testing in the meat control. One practical way to overcome this obstacle would be to use a harmless marker substance, together with chloramphenicol, that easily could be detected at the slaughter. This could be a cheap and simple method to obtain control over possible administration of chloramphenicol to slaughter animals.

Table 1. Biological determination of residues of chloramphenicol in kidney tissues after an intramuscular injection in pigs.

Pig	Inhibition zone in millimeters using			
	M. luteus	B. subtilis BGA	B. subtilis ATCC 6633	B. stearothermophilus
15 mg/kg 4 h	4	-	4	6
20 mg/kg 3 h	1-2	1-2	1-2	8
"- 3 h	0	1-2	1-2	8
"- 6 h	0	1-2	3	8
"- 6 h	0	1-2	3	8
"- 18 h	0	1-2	3	8
18 h	0	1-2	3	8
25 mg/kg 1 h	3	3	3	5
35 mg/kg 1 h	4	3	3	6
Control	0	1-2	0	8

Table 2. Biological determination of residues of chloramphenicol in urine after an intramuscular injection in pigs.

Pig	Inhibition zone in millimeters using			
	M. luteus	B. subtilis BGA	B. subtilis ATCC 6633	B. stearothermophilus
15 mg/kg 4 h	2	3	12	10
20 mg/kg 3 h	1-2	0	0	0
"- 6 h	5	6	10	7
"- 6 h	6	6	9	6
"- 18 h	0	0	0	0
18 h	0	0	0	0
25 mg/kg 1 h	9	9	10	9
35 mg/kg 1 h	-	10	-	8
Control	0	0	0	0

References

English, P B and Withy, D, 1959. Aust. Vet. J. 35, 187.

English, P B and Seawright, A A, 1961. Aust. Vet. J. 37, 9.

Fabianson, S, Nilsson, T and Bäckström, J, 1976. To be published.

Forschner, E and Glende, W, 1976. Die Fleischwirtschaft 2, 226.

Grove, D C and Randall, W A, 1955. Assay methods of antibiotics: a laboratory manual. Medical Encyclopedia Inc. New York.

Lammers, W, Nelemans, F A and Siderius, P, 1961. Algemene Farmacotherapie, Staflen, Leiden.

Oh-Ishi, S, 1968. Jap. J. Vet. Sci. 30, 25.

Pauli, J V and English, P B, 1971. J. Small Anim. Pract. 12, 643.

Schwank, R and Jirasek, L, 1963. Hautarzt 14, 24.

Sisodia, C S, Dunlop, R H, Gupta, V S and Taksas, L, 1973. Am. J. Vet. Res. 34, 1147.

Watson, A D J, 1972. J. Small Anim. Pract. 13, 147.

Wintrobe, M M, 1961. Clinical Hematology. Henry Kimpton, London, 5th ed.

Ziv, G, Bogin, E and Sulman, F G, 1973. Zbl. Vet. Med. 20, 801.