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Institute for Cardiovascular Research, Prague 4-Krč; Institute of Clinical and Experimental Surgery, Prague 4-Krč; Department of Pathology, Paediatric Medical School of the Charles University, Prague (Czechoslovakia)

Chlortetracycline and Oxytetracycline in Experimental E. coli Pyelonephritis

By

VLADIMÍR PRÁT, MILAN HATALA and DAGMAR BENEŠOVÁ

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Tetracycline antibiotics are frequently used in E. coli pyelonephritis in man. Their different derivatives have a similar antimicrobial action *in vitro*, differ however, as regards their circulation in the organism; differences were found in their penetration into different tissues and body fluids as well as in the rate and routes of their excretion from the organism. These facts may exert an important influence on the therapeutic effectiveness of different tetracycline antibiotics in localized infections such as pyelonephritis. More detailed data in this respect are lacking. We decided therefore to obtain some information on the model of bacterial inflammation of the kidneys, produced by the most frequently pathogenetic microorganism of human pyelonephritis, Escherichia coli. In the present communication we shall pay attention to a comparison of the effect of chlortetracycline (CTC) and oxytetracycline (OTC).

Data published in the literature on these two tetracycline antibiotics suggest some important differences as regards their circulation in the organism:

a) the kidneys excrete 70% of the amount of OTC administered but only 18% CTC (KUNIN et al., 1; KUNIN 2).

b) the OTC concentration in urine is many times greater than the CTC concentration,

c) their distribution in renal tissue under physiological conditions is different as proved by MÁLEK et al., (1, 2), using the fluorescence method; CTC accumulates more in the renal cortex, while CTC accumulates in the medulla.

We tried to test whether the above differences between CTC and OTC in relation to the kidneys will be manifested in their therapeutic effect on the course of experimental E. coli pyelonephritis.

Methods

The experiments were carried out on full grown male rabbits, chinchilla strain, weighing 2,500 to 3,500 g. The basic experimental set-up was as follows: induction of acute haematogenous E. coli pyelonephritis, two weeks' administration of CTC or OTC and sacrificing of the animals one week after termination of treatment, with subsequent histological examination of the kidneys and bacteriological examination of renal tissue and urine.

A suspension of the microorganisms in physiological saline was prepared in a 18-hour agar medium with the haemolytic variant of the E. coli strain, serotype 026 : B 6; every animal was injected with approximately $5 \cdot 10^8$ live microorganisms. The strain used was sensitive to tetracycline antibiotics in concentrations of 10 gamma or more (disc-method).

Left-sided acute pyelonephritis was induced by the i.v. injection of the suspension to animals with a temporary ligature of the left ureter the passage through which was completely renewed after removal of the ligature; this method has been used in our laboratory for several years (PRÁT et al.).

Chlortetracycline (Aureomykoin-Spofa) was administered twice a day intravenously in amounts of 10 mg/kg body-weight per injection, oxytetracycline (Oxymykoin-Spofa) twice a day intramuscularly in amounts of 15 mg/kg body-weight per injection. The amounts of antibiotic correspond to double the amount used in the treatment of young children. Different routes of administration were used because there exists no intramuscular CTC preparation and intravenous OTC preparations cannot be used for prolonged periods as they cause, as we found, very soon severe thrombophlebitis. Treatment was initiated either on the day when the animals were injected with microorganisms ("early" treatment) or two days later ("delayed" treatment).

The animals were killed by i.v. administration of a pentothal solution one week after termination of the two-week therapy. During necropsy the abdominal cavity was opened under sterile conditions and first of all urine for bacteriological examination was withdrawn from the bladder by puncture. Next both kidneys and ureters and the urinary bladder were removed. The lower $^2/_3$ of the kidneys with the ureter was subjected to histological examination, the upper $^1/_3$ to bacteriological examination. Urine and renal tissue were examined by a quantitative bacteriological dilution method (pour plate method) and the number of microorganisms per ml of urine or gram of homogenised renal tissue was assessed. In all positive E. coli cultures a serological reaction using the method of cover slip agglutination with specific anti-OB serum was made to establish the identity of the detected E. coli strain with that used to produce pyelonephritis.

In three control groups the histological and bacteriological findings were evaluated in untreated rabbits three weeks after producing unilateral acute pyelonephritis, and in non-infected rabbits to whom for a period of two weeks CTC or OTC was administered after a temporary ligature of the left ureter, and finally in non-infected rabbits after ligature of the left ureter to whom antibiotics were not administered.

The characteristics of individual experimental groups can be summarized as follows:

1. Temporary ligature of ureter + infection + "early" CTC therapy.
2. Temporary ligature of ureter + infection + "early" OTC therapy.
3. Temporary ligature of ureter + infection + "delayed" CTC therapy.
4. Temporary ligature of ureter + infection + "delayed" OTC therapy.
5. Temporary ligature of ureter + infection without therapy.
6. Temporary ligature of ureter + therapy without infection.
7. Temporary ligature of ureter without infection and without therapy.

Evaluation of results. To render evaluation and comparison of the histological findings of the kidneys possible, it was essential to divide the varied histological changes into three groups:

a) negative finding,
b) focal inflammatory changes, affecting only small circumscribed portions of tissue,
c) diffuse changes affecting the whole organ or its predominant part.

In addition the inflamatory changes were evaluated according to the activity of the process into active (predominance of leucocytes incl. focus of abscess), and inactive (post-inflammatory scarring). Microscopic changes caused by temporary congestion of urine during temporary ligature of the ureter without infection were rated as negative.

The histological findings on the right kidney with the non-ligatured ureter were only in rare instances positive in animals of the infected groups, i.e. small inflammatory foci were found; in view of the insignificant and rare occurrence of these changes, the findings on the right kidney are not evaluated in the results. The bacteriological findings were also evaluated in the left kidney only, as on the right side in all instances — even in the infected groups — they were negative as far as the presence of significant numbers of E. coli is concerned.

In the preliminary experiment in rabbits CTC and OTC concentrations in blood, urine, renal cortex and medulla were assessed after a single i.v. injection (CTC) or i.m. injection (OTC) in amounts corresponding in individual rabbits to the amount used in the experiment proper.

Results

1. CTC and OTC levels in blood, urine and kidneys following a single dose. Tables 1 and 2 show above all that there exist differences in the CTC and OTC concentration of the cortex and renal medulla. CTC reaches higher concentrations on the cortex, while the OTC concentrations are higher in the medulla. There are also very striking differences between the CTC and OTC concentration of the bladder urine, the concentration of OTC being much higher than that of CTC. The blood levels can be hardly compared in view of the different route of administration used.

Table 1. *Blood, urine and kidney tissue levels of chlortetracycline (35 mg of chlortetracycline [Aureomykoin Spofa] intravenous, male-rabbits, 2,600—3,100 g body weight)*

Time after injection	Blood γ/ml	Urine γ/ml	Kidney (γ/g)	
			Cortex	Medulla
3 hrs	5.7	32.5	72.0	9.0
	2.04	27.5	65.0	9.6
	2.94	19.5	52.0	11.6
	4.5	27.5	39.0	13.6
	1.86	82.0	14.8	6.8
	3.41 ± 1.65	33.8 ± 25.14	48.6 ± 22.71	10.1 ± 2.59
6 hrs	0.465	20.5	9.2	0.98
	0.555	74.0	5.4	2.1
	0.315	22.0	4.6	1.96
	0.435	31.0	6.5	2.4
	0.360	44.0	15.6	4.6
	0.426 ± 0.025	38.3 ± 22.0	8.26 ± 4.46	2.41 ± 1.34
12 hrs	0.198	6.6	1.04	0.36
	0.216	8.8	1.56	0.39
	0.165	8.8	0.78	0.33
	0.165	6.6	3.1	1.1
	0.460	—	3.1	0.92
	0.242 ± 0.124	7.7 ± 1.3	1.92 ± 1.12	0.62 ± 0.36

Statistical analysis of CTC levels between cortex and medulla: after 3 hrs — $t_f = 3.364$ $P < 0.05$, after 6 hrs — $t_f = 2.515$ $P > 0.05$, after 12 hrs — $t_f = 2.208$ $P > 0.05$.

2. Histological finding in the kidneys. The results in treated rabbits (Table 3) indicate, that "early" treatment, i. e. on the day of injection of the microorganisms can prevent in approximately 85% of the animals the development of pyelonephritis, there being no difference between CTC and OTC in this respect; rarely encountered pyelonephritic changes (ca 15%) were only of a focal type and none of the rabbits died. Substantially different results were obtained in rabbits where treatment was initiated two days after injection of the microorganisms. Only 21.9% after CTC and 31.6% after OTC treatment did not develop pyelonephritis; more than 50% of the rabbits developed severe diffuse inflammatory changes in the kidney with persisting activity of the process. Somewhat less satisfactory were the results of histological examinations of kidneys from rabbits

Table 2. *Blood, urine and kidney tissue levels of oxytetracycline (40 mg oxytetracycline [Oxymykoin Spofa] intramuscular, rabbits-male 2,600—3,100 g body weight)*

Time after injection	Blood γ/ml	Urine γ/ml	Kidney (γ/g)	
			Cortex	Medulla
3 hrs	2.8	1,400	5.4	26.0
	0.81	0,700	9.4	22.0
	1.65	2,000	16.0	32.0
	0.87	2,800	16.0	44.0
	1.53 ± 0.93	1,725 ± 892	11.7 ± 5.2	31.0 ± 9.6
6 hrs	1.92	0,790	6.4	24.0
	3.15	1,600	4.5	34.0
	1 62	1,200	12.6	34.0
	1.44	0,950	5.4	21.0
	1.92	1,000	9.0	24.0
	2.01 ± 0.67	1,108 ± 312	7.58 ± 3.27	27.4 ± 6.15
10 hrs	3.6	400	9.0	34.0
	1.35	200	9.0	18.0
	1.62	140	18.0	84.0
	1.35	200	9.0	24.0
	0.66	70	12.6	30.0
	1.72 ± 1.11	202 ± 123	11.5 ± 3.94	38.0 ± 26.42

Statistical analysis of OTC levels between cortex and medulla: after 3 hrs — $t_f = 3.059$ $P < 0.05$, after 6 hrs — $t_f = 4.452$ $P < 0.005$, after 10 hrs — $t_f = 1.982$ (insignif.).

Table 3. *Results of histological examinations*

Experimental group	No. of animals	Without inflammatory changes	Focal inflammatory changes	Diffuse inflammatory changes	Active suppuration	No. of animals died of sepsis
CTC — "early" therapy .	14	12= 85.7%	2=14.3%	0	1= 7.1%	0
OTC — "early" therapy .	13	11= 84.6%	2=15.4%	0	1= 7.7%	0
CTC — "delayed" therapy	32	7= 21.9%	7=21.9%	18=56.2%	21=65.6%	4=12.5%
OTC — "delayed" therapy	19	6= 31.6%	3=15.8%	10=52.6%	11=57.9%	1= 5.3%
Controls: Infected animals without therapy .	30	5= 16.7%	2= 6.6%	23=76.7%	23=76.7%	8=26.7%
Uninfected animals with therapy . .	9	9=100.0%	0	0	0	0
Unifected animals without therapy (temporary ureteral ligature only) . .	9	9=100.0%	0	0	0	0

Statistical analysis: differences in occurence of active renal suppuration between CTC or OTC "delayed" to infected controls without therapy not statistically significant.

subjected to CTC therapy; the differences were however not statistically significant. The lower effectiveness of CTC is suggested also by the fact that in this group four rabbits (12.5%) died of septicaemia (perinephritis and peritonitis) while in the group treated with OTC only one rabbit (5.3%). As compared with the control group of 30 untreated rabbits, the results as regards the frequency and extent of inflammatory changes in the kidney of rabbits treated with CTC are only little more favourable and the differences are not statistically significant. In all rabbits of the non-infected control groups the histological findings, as far as the incidence of inflammatory changes is concerned, were negative in all instances.

3. Bacteriological changes. Bacteriological examinations of the left kidney after sacrificing the animals (Table 4) revealed that "early" CTC or OTC treatment was able to sterilize in all instances the renal tissue after a previous temporary block of the ureter. Also "delayed" OTC treatment eliminated in all animals microorganisms from the renal tissue. On the other hand, after "delayed" CTC therapy a positive bacteriological finding persisted in the renal tissue of 11 from a total of 32 rabbits (34.4%); in most instances the concentration of microorganisms was high — above 10^3/g. From a total of 30 rabbits of the control group without treatment the kidneys were infected in 20 (66.7%). In all animals with a finding of this high concentration of microorganisms in the tissue (even above 10^3/g) also considerable bacteriuria with E. coli concentrations of above 10^4/ml urine were found; in one rabbit after "delayed" CTC therapy considerable bacteriuria was found in the absence of a positive finding in renal tissue. This case

Table 4. *Results of bacteriological examination*

Experimental group	No. of animals (100%)	Bacteriology of renal tissue			Bacteriuria (above 10^4/ml without positive tissue)	Total No. of	
		negative	below 10^3/g	above 10^3/g		negative	positive
CTC — "early" therapy	14	14=100.0%	0	0	0	14=100.0%	0
OTC — "early" therapy	13	13=100.0%	0	0	0	13=100.0%	0
CTC — "delayed" therapy	32	21= 65.6%	3=9.4%	8=25.0%	1=3.1%	20= 62.5%	12=37.5%
OTC — "delayed" therapy	19	19=100.0%	0	0	0	19=100.0%	0
Controls: Infected animals without therapy	30	10=33.3%	9=30.0%	11=36.7%	0	10= 33.3%	20=66.7%

Statistical analysis: CTC — "delayed" to Controls: insignif. Comparison of CTC — "delayed" to other groups and Controls to other groups statistically signif.

must be also added to the total number of infected animals as it suggests the presence of an inflammatory process localised in the efferent urinary pathways but not affecting the renal parenchyma. The summarized results indicate that the total number of rabbits with persisting E. coli infection in the group of rabbits after "delayed" CTC therapy is 12 from a total number of 32 (37.5%) and in the untreated control group 20 from a total of 30 (66.7%).

4. Changes in body-weight of rabbits in the course of the experiment. The suppurative inflammatory renal process exerts negative influence on the metabolism of experimental animals; the weight-loss during the experiment thus gives a rough idea on the severity of the inflammatory process. The average weight changes in individual experimental groups (Table 5) indicate that in non-infected controls the body-weight declines during the three weeks of observation only slightly, by 1.9 to 2.14%. In rabbits with "early" CTC or OTC treatment the weight-losses are greater, by 6.31 to 7.33%. The greatest body-weight loss 14.06% was noted in rabbits with "delayed" CTC therapy; on the other hand in rabbits with "delayed" OTC therapy the body-weight declined only by 9.98%. The decline of body-weight in infected non-treated controls was by 11.41% lower than after "delayed" CTC therapy.

Table 5. *Changes in body-weight during experimental period (i.e. differences between the original weight and the weight before sacrifice)*

Experimental group	No. of rabbits	Mean change of body weight (in g)	Mean % change of original body weight
1. CTC — "early" therapy . .	13	— 236	— 7.33
2. OTC — "early" therapy . .	14	— 173	— 6.31
3. CTC — "delayed" therapy .	32	— 484	— 14.06
4. OTC — "delayed" therapy .	19	— 311	— 9.98
Controls:			
5. Infection without therapy .	30	— 321	— 11.41
6. Therapy without infection .	9	— 61	— 1.91
7. Temporary ureteral ligature without therapy and without infection	9	— 72	— 2.14

Statistically significant differences:

3 to 6: $t=3.319$	$p<0.005$	4 to 7: $t=2.885$	$p<0.01$
3 to 7: $t=2.625$	$p<0.025$	5 to 6: $t=3.684$	$p<0.01$
6: $t=3.154$	$p<0.005$	5 to 7: $t=3.362$	$p<0.005$

Discussion

In the literature repeatedly evidence was provided that there exist differences between the renal cortex and medulla as regards susceptibility to infection, the medulla being less resistant, and even a small amount of microorganism produces an inflammation (ROCHA et al., GUZE et al., 1). In the medulla microorganisms may persist after completed therapy of experimental enterococcal pyelonephritis and they may be the source of a subsequent exacerbation of an inflammatory process (GUZE et al., 2). It may be thus assumed that drugs with a greater affinity for the renal medulla are more effective in the treatment of pyelonephritis than

drugs lacking this property. Comparison of the concentrations in the cortex and medulla confirmed the data of MÁLEK et al., (1, 2) that OTC reaches higher concentrations in the medulla, while CTC reaches higher levels in the cortex.

The results of our experiments where the effectiveness of CTC and OTC in acute obstructive E. coli pyelonephritis was compared suggest in several respects a greater effect of OTC as compared with CTC.

In experiments where CTC or OTC therapy was initiated simultaneously with the administration of E. coli to the organism ("early" therapy) the differences between the two tetracycline antibiotics were not manifest and it proved possible in 85% of the animals to prevent the development of pyelonephritis; not in a single case E. coli were found in renal tissue or urine. The results provide evidence of the importance of early treatment. Similar favourable results in early therapy of enterococcal pyelonephritis were obtained by FISHER et al., contrary to the unfavourable results obtained when treatment was initiated only several days after the development of the infection. The differences between CTC and OTC became manifest only in rabbits where treatment was initiated as late as two days after injection of the microorganisms. At that time the microorganisms had already settled down and reproduced in the renal tissue and produced an acute suppurative inflammation and caused in most animals irreparable morphological changes. From the histological aspect there was no significant change in the extent and activity of the persisting inflammatory changes though after OTC treatment these changes were less frequent. The bacteriological examination of the kidneys revealed, however, marked differences between the two antibiotics; OTC removed the microorganisms from the renal tissue in 100% of all rabbits, while CTC did so only in 62.5%. The lower effectiveness of CTC is suggested also indirectly by the somewhat greater number of animals who died of septicaemia because the suppurative inflammations spread to the peritoneum, and by the greater body-weight loss in the course of the experiments.

Our results indicate thus that OTC is probably a more effective means in the treatment of E. coli pyelonephritis than CTC.

These findings require, of course, confirmation by further experiments and comparison with other tetracycline antibiotics.

Summary

On experimental haematogenous E. coli pyelonephritis in rabbits produced by temporary ligature of the ureter the effect of chlortetracycline (CTC) and oxytetracycline (OTC) was compared. When the treatment was initiated "early" (i.e. on the day when the microorganisms were injected), the development of the inflammatory process could be prevented in 85%; no marked differences were found between the effect of CTC and OTC. When "delayed" treatment was applied (i.e. two days after injection of the microorganisms) no marked inflammatory changes developed in the kidneys of 22% of the CTC-treated rabbits and in 32% of the OTC-treated rabbits; after OTC therapy the renal tissue was always sterile, after CTC therapy positive bacteriological findings persisted in 37,5%.

Beeinflussung der experimentellen Escherichia coli-Pyelonephritis durch Chlortetracyclin und Oxytetracyclin

Zusammenfassung

In Versuchen an Kaninchen wurde durch zeitweilige Ligatur eines Ureters und intravenöse Injektion von Escherichia coli eine Pyelonephritis erzeugt und ihre Beeinflussung durch Chlortetracyclin (CTC) und Oxytetracyclin (OTC) verglichen. Wenn die Behandlung früh einsetzte (d.h. am Tage der Injektion der Mikroorganismen), konnte man die Entwicklung des entzündlichen Prozesses in 85% verhindern; dabei wurde kein auffälliger Unterschied zwischen CTC und OTC gefunden. Wenn die Behandlung dagegen später einsetzte (2 Tage nach Injektion der Mikroorganismen), entwickelten sich keine auffälligen Veränderungen in den Nieren von 22% der mit CTC behandelten Tiere und in 32% der mit OTC behandelten Tiere. Nach CTC-Behandlung war das Nierengewebe immer steril, nach OTC-Behandlung blieb der bakterielle Befund in 37,5% positiv.

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Dr. med. V. PRÁT
Prag 4-Krč, Budějovická 809 (ČSSR)
Institut für Kreislaufforschung