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Pharmacokinetics and tissue localization of doxycycline polyphosphate and doxycycline hydrochloride in the rat

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SUMMARY

Two doxycycline derivatives Doxycycline polyphosphate and Doxycycline hydrochloride were administered to rats at a dose of 20 mg/kg body weight. Doxycycline tissue levels were determined using a microbiological assay.

Only an insignificant fraction of the antibiotics was found to cross the blood brain barrier. Doxycycline was highly concentrated in excretory organs : liver, kidneys and caecum. The high intestinal drug level observed is probably related to the entero-hepatic cycle of this antibiotic.

There was a good correlation between serum and heart doxycycline concentration ; heart level was about twice that of serum. In lung, antibiotic level was always higher than in serum.

INTRODUCTION

Although pharmacokinetics of antibiotics have been widely studied, their extra-vascular transfer and tissue distribution are still more or less unknown.

It is generally assumed that the distribution and penetration of antibiotics in the tissues and interstitial fluids depend on the free antibiotic concentration in the central compartment (1). As a rule antibiotics that are more than 80% bound to serum proteins should have a very low distribution in the body (2).

Doxycycline, a second generation tetracycline, falls into this category but nevertheless has extensive tissue distribution. Doxycycline is highly liposoluble, or more accurately has a high relative liposolubility which readily compensates for the high protein binding (3).

The present study is a comparison, in the rat, of pharmacokinetic parameters and tissue localization determined for doxycycline polyphosphate (DPP) and doxycycline hydrochloride (DHC).

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MATERIALS AND METHODS

The studies were performed using male and female « Wistar » rats weighing 200 g ± 50 g.

The two antibiotics under test were sodium doxycycline polyphosphate (DPP), batch 6603 and doxycycline hydrochloride (DHC). The drugs were administered at a dose of 20 mg/kg body weight (calculated as doxycycline base). This dosage was selected in order to obtain serum levels similar to those observed in man after a therapeutic dose.

The rats were fasted for 18 hours before the experiment in order to increase the bioavailability of the antibiotic (4). The two doxycycline salts were administered (in a 400 mg/l aqueous solution) by gastric tube. The animals were then sacrificed by carotid section, in groups of 10, 1, 2, 3, 4, 6, 12 and 24 hours after administration of the drugs.

After coagulation of blood samples, the serum was stored at -24°C until the assay.

Organs : brain, lungs, heart, liver, kidneys and intestine (caecum) were sampled upon dissection, washed in isotonic solution in order to reduce blood contamination, carefully drained then stored frozen at -40°C until the determination.

The determinations were carried out using the agar diffusion technique (5) with Bacillus subtilis ATCC 6633 as the test organism.

A preliminary study allowed the selection of a common extraction method for all the homogenates of tested organs. To ensure homogenization we used ultrasonication for the following times; 30 seconds for the brain, 1 min. for the kidneys and liver, 2 min. for the lungs and heart. The intestinal contents, diluted and homogenized using a Vortex mixer, were centrifuged for 5 minutes at 6000 rev./min. to reduce bacterial contamination, which, if extensive, would lead to inaccurate results.

Control preparations were made for each organ following the same protocol: organs and serum were sampled from untreated rats. After homogenization exact quantities of antibiotics were added for the following ranges:

Serum: 0.25 - 0.5 -1 - 2 - 4 mg/l

Caecal contents: 24 - 48 - 96 - 192 - 384 mg/l

Various organs: 3 - 6 - 12 - 24 - 48 mg/l.

In order to select the more appropriate extraction method, we compared the regression curves ($y = a + bx + cx^2$) obtained with standard solutions made either in distilled water, organ homogenates or in various extraction fluids. The determinations were performed either on the supernatant extraction fluid after centrifugation, or on the suspension obtained after ultrasonication. For two volumes of extraction medium one volume of organ was used.

We selected distilled water as extraction fluid, because it well preserves the antibiotic which is sensitive to alkaline pHs, and can possibly lead to cell lysis, releasing a maximum amount of antibiotic.

The results were calculated using the third statistical method given by Bennet, Brodie, Benner and Kirby (5). Once the regression curve coordinates $y = a + bx + cx^2$ describing O = f log dose (O = inhibition zone diameter) are calculated, we can, instead of considering this curve as being a straight line, introduce an assay mean (m) into the formula.

$$X = \sqrt{\frac{b^2 - 4ac + 4cm}{2c}} - b$$

Thus, the concentration of this assay e is:

$$e = \text{minimum conc. of the range } X \frac{\text{antilog } (0.3010\ X)}{2}$$

This method markedly reduces the standard deviation of the results.

RESULTS

As a general rule, the results:

< 0.2 mg/l or kg for serum, brain, lungs and heart

< 0.5 mg/kg for liver

<0.7 mg/kg for kidneys, and caecum, must be interpreted as being traces of antibiotics. This follows on from the limits of accuracy of the determinations.

The concentration of doxycycline in various tissues of rats treated with doxycycline polyphosphate (DPP) or doxycycline hydrochloride (DHC) are shown in Tables I and II.

In the serum, the only significant difference between the two treatments was the concentration 2 hours after administration which proved to be higher for DPP (2.81 mg/l) than for DHC (1.78 mg/l) with a probability threshold of 0.05.

The intracerebral concentration (Tables I and II) was the same for the 2 drugs. Only a very small fraction of each salt crosses the blood-brain barrier and only traces of antibiotic were detected in the brain; this observation agrees with clinical observations reported by Mingat (6).

Levels in the lung (Tables 1 and II) are, in many animals, higher than in the serum. There is, however, no significant correlation between serum and lung concentration.

1 hour after administration of the drug, DPP produces significantly higher levels in lung tissue than DHC does, with a probability threshold of < 0.001.

6 hours after ingestion of DPP, lung levels are also higher ($p < 0.02$) than after DHC.

Concentrations of doxycycline obtained in the cardiac muscle are shown in Tables I and II. There is a good correlation between serum and cardiac levels for both drugs. The ratio between tissue and serum concentration is 2.23 for DPP and 2.55 for DHC. DHC produces higher cardiac levels after 12 hours ($p < 0.001$) and after 24 hours ($p < 0.01$) than DPP does.

In excretory organs (liver, kidneys, intestine) antibiotic concentrations are greater than in plasma (Tables I and II). Liver levels 1 hour ($p < 0.01$) and 2 hours ($p < 0.05$) following ingestion of DPP are higher than after DHC. At 6 hours and 24 hours however, DHC gives higher liver concentrations ($p < 0.01$) than DPP does.

In kidney tissue, DHC produces higher levels than DPP 6 hours ($p < 0.001$), 12 hours ($p < 0.05$) and 24 hours ($p < 0.5$) after administration.

DISCUSSION AND CONCLUSIONS

From the experimentally measured concentrations in serum and in various organs, values were determined for the principal pharmacokinetic parameters of the 2 doxycycline salts.

Table I: Rat tissue concentrations (mg/kg) after oral administration of DPP

TISSUE \ TIME	1 h	2 h	3 h	4 h	6 h	12 h	24 h
SERUM	1.80 ± 0.95	2.81 ± 1.20	2.46 ± 0.97	1.90 ± 0.66	1.12 ± 0.58	0.44 ± 0.39	0.22 ± 0.12
BRAIN	0.26 ± 0.12	0.21 ± 0.08	0.18 ± 0.05	0.22 ± 0.07	0.20 ± 0.07	0	0
LUNG	3.11 ± 0.76	3.88 ± 1.98	3.10 ± 1.76	3.25 ± 1.03	2.38 ± 0.89	1.18 ± 0.52	0.39 ± 0.16
HEART	4.40 ± 3.71	5.80 ± 2.31	4.95 ± 3.44	4.00 ± 2.84	2.51 ± 1.27	0.89 ± 0.32	0.30 ± 0.10
LIVER	33.10 ± 14.64	34.15 ± 15.35	21.00 ± 7.11	18.50 ± 9.09	11.62 ± 2.63	5.21 ± 1.46	3.31 ± 0.77
KIDNEY	30.75 ± 15.01	38.56 ± 9.74	42.10 ± 12.39	39.94 ± 5.01	21.05 ± 5.01	8.10 ± 3.33	3.07 ± 0.89
CAECUM	3.00 ± 1.40	5.95 ± 2.05	15.10 ± 8.92	79.20 ± 22.26	70.00 ± 26.15	42.90 ± 15.15	19.80 ± 6.65

Table II: Rat tissue concentrations (mg/kg) after oral administration of DHC

TISSUE \ TIME	1 h	2 h	3 h	4 h	6 h	12 h	24 h
SERUM	1.58 ± 0.75	1.78 ± 0.65	1.87 ± 0.68	1.65 ± 0.46	1.34 ± 0.27	0.35 ± 0.15	0.24 ± 0.07
BRAIN	0.23 ± 0.07	0.18 ± 0.07	0.14 ± 0.09	0.16 ± 0.15	0.17 ± 0.13	0.13 ± 0.08	0
LUNG	1.91 ± 0.56	3.05 ± 1.08	3.00 ± 1.53	2.70 ± 1.00	1.59 ± 0.19	0.82 ± 0.58	0.42 ± 0.08
HEART	3.65 ± 1.27	4.46 ± 1.63	4.10 ± 1.64	3.77 ± 1.84	2.81 ± 1.04	1.63 ± 0.49	0.69 ± 0.41
LIVER	14.90 ± 4.82	21.05 ± 6.29	16.00 ± 3.75	17.71 ± 6.08	16.25 ± 3.06	5.70 ± 0.96	5.12 ± 1.14
KIDNEY	42.40 ± 11.17	40.13 ± 9.33	44.06 ± 5.84	41.92 ± 9.39	42.96 ± 12.85	11.80 ± 3.69	4.41 ± 1.79
CAECUM	1.40 ± 0.78	7.80 ± 3.96	21.10 ± 8.89	60.50 ± 18.24	74.90 ± 23.84	34.60 ± 22.87	25.10 ± 11.79

Bearing in mind the small number of experimental values we considered that the 2 drugs folwed an open one compartment kinetics. The equations describing the kinetics are thus of the type:

$$C = -Ae^{-\alpha t} + Be^{-\beta t}$$

We calculated the various equations using a Digital PDP 11 computer and the results obtained are as follows:

SERUM
$$DPP: C = -8.24e^{-0.99t} + 6.62e^{-0.28t} \quad (1)$$
$$DHC: C = -3.67e^{-0.84t} + 3.69e^{-0.18t} \quad (2)$$

LUNGS
$$DPP: C = -4.99e^{-1.40t} + 4.97e^{-0.12t} \quad (3)$$
$$DHC: C = -6.19e^{-0.75t} + 6.12e^{-0.18t} \quad (4)$$

HEART
$$DPP: C = 11.78e^{-0.95t} + 11.73e^{-0.24t} \quad (5)$$
$$DHC: C = -5.73e^{-1.41t} + 5.71e^{-0.10t} \quad (6)$$

LIVER
$$DPP: C = 52.39E^{-2.11t} + 51.50e^{-0.25t} \quad (7)$$
$$DHC: C + 25.42e^{-1.21t} + 25.41e^{-0.09t} \quad (8)$$

KIDNEYS
$$DPP: C + 141.55e^{-0.59t} + 141.33e^{-0.26t} \quad (9)$$
$$DHC: C = 68.11e^{-1.05t} + 68.92e^{-0.12t} \quad (10)$$

No equation has been calculated for doxycycline's kinetics in the brain and in the caecum.

The pharmacokinetic parameters calculated from equations 1 to 10 are shown in Tables III and IV.

It should be noted that the half-lives are probably longer than the open one compartment model would suggest, as the calculated and the experimental values became different by the twelth hour. Because of the lack of concentration values corresponding to time intervals between 12 and 24

Table III: Rat serum pharmacokinetic parameters after oral administration of 20 mg/kg of DPP or DHC

	α h^{-1}	t 1/2 α h	β (h^{-1})	t 1/2 β h	Vd 1/kg	AUC h./mg/l	Cl 1/h/kg	C max. mg/l	t max h
DPP	0.99	0.70	0.28	2.47	4.56	15.50	1.29	2.64	2.09
DHC	0.84	0.82	0.18	3.85	6.88	16.21	1.23	1.91	2.33

Table IV: Rat tissue pharmacokinetic parameters

TISSUE	C max. mg/l		t max. (h)		t 1/2 β (h)	
	DPP	DHC	DPP	DHC	DPP	DHC
LUNG	3.62	2.91	1.93	2.50	5.85	3.85
HEART	5.44	4.29	1.93	2.00	2.89	6.93
LIVER	34.07	18.95	1.16	2.29	2.79	7.70
KIDNEY	41.48	46.73	2.47	2.35	2.67	5.78
SERUM	2.64	1.91	2.09	2.33	2.48	3.85

hours, we could not use an open two compartment model which would probably have better described the observed effects.

Initial analysis shows that the results are quite heterogeneous taking into account the differences in behaviour from animal to animal, a fact which has already been emphasized in previous pharmacokinetic studies on tetracyclines (7). A recent study of doxycycline pharmacokinetics in the rat (8) showed that the difference between antibiotic concentrations in different animals was more or less great depending to the tissue; however this difference did not exceed 1/3 of the mean of a group in the case of non-excretory organs even though the variability of blood contamination (evaluated by Fabre (8) from 0.5 to 1.5% of the tissue weight and by Campistron (9) from 0.2% in muscle to 31% in kidneys).

In kidney, because of the presence of residual urine, the « tissue » level of doxycycline measured is highly debatable. Fabre (8) estimates that almost 10% of doxycycline measured in the renal cortex and 30% in the medullary part should be attributed to urine and not to renal tissue.

Concentrations in heart and lungs were always higher than in serum which corroborates Fabre's (8) and Cahen's results (19). The penetration of doxycycline into bronchial secretions has also been demonstrated in man (11, 12, 13) Hartnett (12) has shown that doxycycline remains in sputum for several days after the completion of treatment.

These observations are of great interest in therapeutics.

The liver, kidneys and caecum (excretory organs) were particularly rich in antibiotics, as already emphasized by several authors (14, 15, 10, 8). The storage of an active form in the liver might explain the entero-hepatic cycle reported by Schach Von Wittenau (16, 17) and by Fourtillan (18).

The amount of doxycycline observed in the caecum could explain the non-accumulation of the antibiotic in patients with renal failure (19, 20). In these patients, intestinal excretion subtitutes for the low renal elimination (21, 22).

Regardless of the salt given, doxycycline levels were, except for brain, always higher in tissues than in serum (Tables I and II).

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