Disease states may change the normal function of the body and may alter the pharmacokinetics of several different drugs (Benet, 1976). The incidence of infections caused by gram-negative bacteria is high and these disease states pose potential therapeutic problems if the diseases alter the pharmacokinetics of drugs administered for the treatment of the infections. Because most disease states are complex entities, it is often difficult to properly assign observed pharmacokinetic changes to specific symptoms of the disease. The only way to solve this problem is by using animal models which incorporate specific components of the disease so that the relative contribution of each of the individual components of the disease can be measured. Previously it has been shown that bacterial endotoxins can change the distribution of drugs (Ladefoged, 1977). In endotoxin induced shock in rabbits the pharmacokinetics of warfarin has been shown to be very different from the kinetics in healthy rabbits (Ladefoged, 1978).

The present experiments were undertaken to clarify whether endotoxemia, which is seen in several infectious disease caused by gram-negative bacteria, could alter the renal excretion of drugs. Sulfathiazole was chosen as the test substance because it is known to have a high renal clearance in pigs (Dalgaard-Mikkelsen and Poulsen, 1956). Inulin clearance was used to estimate the glomerular filtration rate (GFR). In addition the urinary concentration of prostaglandin E was determined before and after endotoxin administration.

Material and Methods

The experiments were performed on seven unanaesthetized, clinically healthy female pigs (Danish Landrace) weighing 16.5—28.0 kg. Each experiment included at least 6 periods of 20 min. duration, 3 control periods and 3 periods with infusion of endotoxin. After a priming dose of inulin (40 mg./kg. b. wt. i. v.) and sulfathiazole (60 mg./kg. b. wt. i. v.) the
animals were infused with inulin and sulfathiazole dissolved in 0.9% NaCl at a rate of 1.5 ml/min. (0.3 mg/kg b.wt./min. for both compounds) through a catheter placed in an ear vein (left side). Endotoxin (E. coli, Sigma®, 1 μg/kg b.wt.) was given intravenously and, subsequently, endotoxin (0.01 μg/kg b.wt./min.) was infused together with inulin and sulfathiazole.

Blood samples were taken through a catheter in an ear vein (right side) 5 min. after the beginning of each period. Urine was collected though a balloon-catheter (Perry FR® 10/3 cc) placed in the bladder. Equilibration periods of 30 min. were allowed before sampling was started after inulin-sulfathiazole-priming and endotoxin-priming.

The pH of the blood and urine samples was measured potentiometrically with a micro-glass electrode (Radiometer) at 37°C. Inulin was estimated by the method described by Brun (1946). Sulfathiazole was determined according to the method of Bratton and Marshall (1939). Protein-binding was determined by ultrafiltration through cellophane membrane (Poulsen, 1956). Body temperature was measured in the rectum with a mercury thermometer. The concentration of prostaglandin E in urine was determined by radioimmunoassay (Clinical Assays Inc. CA 501).

**Results**

The renal clearance of inulin and sulfathiazole of each experiment is shown in Table 1. The results are given as the average of control periods and as the average of periods during endotoxin infusion. The infusion of endotoxin in the dose applied had no influence on clearance of inulin (control 27.7 ml/min./10 kg b.wt.; during endotoxin infusion 26.8 ml/min./10 kg b.wt.), while clearance of sulfathiazole in plasma was reduced markedly (from 19.7 ml/min./10 kg b.wt. to 10.5 ml/min./10 kg b.wt.) (p<0.005). The mean plasma protein-binding of sulfathiazole was 54 per cent of controls and decreased to 45 per cent during endotoxin infusion (p<0.01). Clearance ratios of non-protein-bound sulfathiazole varied from 1.14 to 1.87 in the control periods and from 0.55 to 1.05 during endotoxin infusion (Tab. 1). In the last two columns in Table 1 are shown the clearance ratios of protein-bound and non-protein-bound (Ult.) sulfonamide.

![Fig. 1. Relationship between clearance ratio (non-protein-bound sulfathiazole/inulin) and the pH of the urine in control periods (▲) and in periods with endotoxin infusion (△). The results shown are 3—4 clearance periods before and after endotoxin infusion in each of 7 pigs](image-url)

In control periods the pH of urine varied between 5.1 and 7.6, but these variations had little if any influence on the excretion of sulfathiazole (Fig. 1). During endotoxin infusion the urine pH was a little lower than in control periods (Fig. 1), but the difference in mean pH was not statistically significant (p>0.1). From Fig. 1 it is further seen that the excretion ratio between clearance of the non-protein-bound sulfathiazole and inulin clearance is lower in endotoxin periods than in control periods. When endotoxin was infused the body temperature increased 1—1.5°C in almost all the pigs as shown in Fig. 2. The concentration of prostaglandin
in urine increased after endotoxin infusion and was many times higher than it was in the control urine (Tab. 2). There was no difference in the urine flow rate between control periods and endotoxin periods ($p > 0.1$).

**Discussion**

Injection of endotoxin or live *E. coli* has been shown to influence renal hemodynamics in dogs (Dedichen, 1972; Dale et al., 1976). In the studies mentioned large doses of endotoxin were used and signs of shock were seen. In the present experiment in pigs we used a dose of endotoxin which did not cause shock and which did not impair the inulin clearance (GFR) (Tab. 1). In agreement with the findings of others (Brass, 1963; Van Miert and Frens, 1968), we found that the pig seems to be rather resistant to the effect of lipopolysaccharide from *E. coli*, evaluated by the effect on temperature (Fig. 2).

In spite of the unchanged inulin clearance, endotoxin caused a marked decrease of sulfathiazole clearance.

Fig. 2. Rectal body temperature in 7 pigs during sulfathiazole treatment shown before and after endotoxin injection. At the arrow marked Prime, the priming dose of sulfathiazole was given.
inulin and sulfathiazole in pigs (mean ± s.e.m.)

Table 1

<table>
<thead>
<tr>
<th>Inulin</th>
<th>Clearence (ml/min/10kg)</th>
<th>Sulfathiazole</th>
<th>Clearance ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C_{sulfath.}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ultrafiltrate of plasma</td>
<td>c</td>
</tr>
<tr>
<td>32.5</td>
<td>28.8</td>
<td>18.7</td>
<td>9.3</td>
</tr>
<tr>
<td>27.9</td>
<td>30.6</td>
<td>19.5</td>
<td>16.4</td>
</tr>
<tr>
<td>23.8</td>
<td>17.5</td>
<td>14.0</td>
<td>3.9</td>
</tr>
<tr>
<td>27.4</td>
<td>25.8</td>
<td>20.8</td>
<td>7.1</td>
</tr>
<tr>
<td>30.0</td>
<td>29.5</td>
<td>24.1</td>
<td>15.7</td>
</tr>
<tr>
<td>20.3</td>
<td>26.7</td>
<td>12.9</td>
<td>7.8</td>
</tr>
<tr>
<td>32.2</td>
<td>28.7</td>
<td>27.6</td>
<td>13.1</td>
</tr>
<tr>
<td>27.7</td>
<td>26.8</td>
<td>19.7</td>
<td>10.5</td>
</tr>
<tr>
<td>±1.7</td>
<td>±1.7</td>
<td>±2.0</td>
<td>±1.8</td>
</tr>
</tbody>
</table>

* c = control period
* e = endotoxin period
* paired-t-test

In addition to filtration, both active tubular secretion and back diffusion are involved in the renal handling of sulfathiazole (DALGAARD-MIKKELSEN and POULSEN, 1956). The plasma concentration during endotoxin infusion was well below the T_{max} for sulfathiazole since the sulfathiazole clearance is unchanged in the plasma concentration range 20-100 μg/ml. (DALGAARD-MIKKELSEN and POULSEN, 1956; own observations). The high plasma concentration of sulfathiazole during endotoxin infusion cannot therefore explain the low excretion of sulfathiazole. The back diffusion is influenced by the pH of the urine so that excretion of sulfathiazole will increase with increasing pH (DALGAARD-MIKKELSEN and POULSEN, 1956). The low lipid solubility of sulfathiazole, however, may explain why the urine pH in the present range had no influence on sulfathiazole excretion. It is unlikely that the small urine pH differences between control and endotoxin periods could account for differences in sulfonamide clearance between control periods and periods during endotoxin infusion.

The protein-binding of sulfathiazole is significantly reduced during endotoxin infusion, but this should not decrease the plasma clearance, but rather increase it. The explanation for the marked change in protein-binding might be that endotoxin may induce changes in plasma free fatty acids (FFA)

Table 2

Effect of endotoxin infusion on prostaglandin excretion in urine

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>Prostaglandin concentration</th>
<th>Prostaglandin concentration</th>
<th>Prostaglandin concentration</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>endotoxin</td>
<td>control</td>
</tr>
<tr>
<td>39</td>
<td>520 - 2040</td>
<td>2750 - 5870</td>
<td>440 - 3290</td>
</tr>
<tr>
<td>41</td>
<td>1120 - 4070</td>
<td>6900 - 11950</td>
<td>560 - 1320</td>
</tr>
<tr>
<td>42</td>
<td>1120 - 4070</td>
<td>6900 - 11950</td>
<td>560 - 1320</td>
</tr>
<tr>
<td>46</td>
<td>1120 - 4070</td>
<td>6900 - 11950</td>
<td>560 - 1320</td>
</tr>
<tr>
<td>47</td>
<td>1120 - 4070</td>
<td>6900 - 11950</td>
<td>560 - 1320</td>
</tr>
</tbody>
</table>

Pig no. 33 and 34 not analyzed.
(Hirsch et al., 1964), which is known to influence the protein-binding of drugs (Gugler et al., 1975).

Endotoxin-induced hypotension in the dog seems to be caused by release of prostaglandin (Herman and Vane, 1976) and during endotoxin shock prostaglandins are released from the kidney (Anderson et al., 1975). In the present experiment a significant increase in urinary prostaglandins was seen after endotoxin infusion (Tab. 2). The implication of prostaglandins in renal hemodynamics has been extensively investigated but is still not fully known (McNay, 1977). Prostaglandins seems to be able to mediate a decrease in ratio between blood-flow in the outer cortex and the inner cortex (McGiff et al., 1974; Larsson and Anggård, 1974). The effect of endotoxin on clearance of sulfathiazole might be explained by changes in intrarenal distribution of blood-flow, caused by the release of prostaglandins during the endotoxin infusion (Tab. 2). Hinshaw et al. (1969) have shown that p-aminohippuric acid (PAH) extraction ratios and excretion rates declined in dogs after administration of endotoxin, which is in agreement with our results obtained with sulfathiazole. Hinshaw et al. (1969) also showed that $T_{\text{mPAH}}$ temporarily decreased during endotoxin infusion, suggesting a depression of renal tubular function, primarily because of vascular reactions after the endotoxin infusion.

In conclusion it has been shown that endotoxin infusion decreases clearance of sulfathiazole without changes in GFR. This fact might be explained by changes in intrarenal hemodynamics.

Summary

The renal clearance of sulfathiazole in pigs was shown to be markedly decreased during infusion of $E.\ coli$ endotoxin, while the inulin clearance was unaffected. The effect of endotoxin on sulfathiazole clearance might be explained by intrarenal changes in blood-flow distribution caused by prostaglandin release, since the amount of prostaglandin in the urine was increased significantly during endotoxin infusion.

Acknowledgements

The technical assistance of Mrs. Gerda Larsen is gratefully appreciated. The prostaglandin assays were kindly performed by Dr. J. Ladefoed, Medical Department P, Rigshospitalet, Copenhagen.

The work was supported by the Danish Agricultural and Veterinary Research Council, Grant No. 513-6649.

Zusammenfassung

Einfluß der $E.\ coli$-Endotoxämie auf die renale Sulfathiazol-Clearance des Schweines

Während der Infusion von $E.\ coli$-Endotoxin war die renale Sulfathiazol-Clearance des Schweines deutlich vermindert, die Inulin-Clearance blieb hiervon unberührt. Da der Prostaglandingehalt des Urins während der Endotoxininfusion erhöht war, dürfte die Sulfathiazol-Clearance durch Veränderungen der Blutdurchströmung in den verschiedenen Teilen der Niere, ausgelöst durch das Prostaglandin, verursacht sein.

Résumé

Influence de l'endotoxémie à $E.\ coli$ sur la clearance rénale du sulfathiazol chez le porc

La clearance rénale du sulfathiazol chez le porc fut nettement diminuée durant l'infusion d'une endotoxine de $E.\ coli$. La clearance de l'inuline fut par
Renal Clearance of Sulfathiazole in Pigs with E. coli Endotoxemia

contre inchangée. Etant donné que le taux de prostaglandine de l’urine fut augmenté durant l’infusion d’endotoxine, la clearance du sulfathiazol devrait être provoqué par des modifications du courant sanguin dans les différentes parties des reins dues à la prostaglandine.

Resumen

Influjo de la endotoxia por E. coli sobre el aclaramiento renal de sulfatiazol en el cerdo

Durante la infusión de endotoxina E. coli se hallaba disminuida de forma manifiesta la aclaración renal del sulfatiazol en cerdos, mientras que no era afectado el aclaramiento de la inulina. Ya que se hallaba aumentado significantemente el contenido de prostaglandina en la orina durante la infusión de la endotoxina, el aclaramiento del sulfatiazol se podría explicar por cambios intrarrenales en la distribución de la perfusión sanguínea, desencadenados por la prostaglandina.

References


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