Tissue distribution of penicillin during constant rate infusion in rats at 71 ATA

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Aanderud L, Bakke OM. Tissue distribution of penicillin during constant rate infusion in rats at 71 ATA. Undersea Biomed Res 1985; 12(1):53–58.—Benzyl[14C]penicillin was infused at a constant rate of 100 mg·kg⁻¹ h⁻¹ for 90 min in rats at 1 ATA air for the control group (n = 10) and at 71 ATA He-O₂ for the pressure group (n = 11). The radioactivity was measured in the arterial blood, brain, liver, kidney, muscle, and fat. The mean brain penicillin concentration after 90 min infusion was approximately 20% increased at 71 ATA (P < 0.05), but the brain:blood concentration ratio was not significantly affected. The mean blood, liver, and muscle penicillin content was slightly higher and the mean kidney content was slightly lower at pressure, but no statistically significant changes were observed.

Information on the way high hydrostatic pressure affects the disposition of drugs is still scarce. Knowledge of drug distribution, metabolism, and excretion is fundamental for the evaluation of drug effects at high pressure, an area that carries considerable theoretical as well as practical importance. Data on drug distribution to and effects on the central nervous system are particularly relevant to the use of therapeutic agents at high pressure. A limited number of studies indicate that drug kinetics may be altered by exposure to increased hydrostatic pressure (1–8), whereas others fail to demonstrate any effect of high pressure on drug metabolism (9–11). The present investigation deals with the distribution of [14C]-labeled benzylpenicillin to the brain, liver, kidney, muscle, and fat in the rat at 71 ATA He-O₂.

MATERIALS AND METHODS

Drug administration

The experiments were designed to compare the tissue and blood concentrations of benzylpenicillin in a steady state condition at 1 and 71 ATA. Male Wistar rats weighing 230–300 g
were used. The control experiments \( (n = 10) \) were carried out in 1 ATA air, and the pressure experiments \( (n = 11) \) were performed at 71 ATA He-O\(_2\).

Benzy1 \(^{14}C\)penicillin potassium (Amersham Int., Amersham, Bucks., England) with a specific activity of 5.33 MBq/mg was dissolved in 0.9% NaCl containing unlabeled penicillin and frozen in plastic vials each containing 925–427 kBq (25–125 μCi). The vials were thawed immediately before use, and diluted with 3 ml 0.9% NaCl containing unlabeled penicillin to yield 100 mg · kg\(^{-1}\) · h\(^{-1}\) using a constant rate infusion pump (Fysiologiska Institutionen, Gothenburg, Sweden), delivering 1.9 ml/h via a Plexiglas syringe. The drug was infused through an indwelling catheter in the femoral vein, and the blood samples were collected from the tail artery.

The animals were killed after 90 min infusion by i.v. injection of saturated KCl solution (control group), or by rapid decompression (pressure group). In 6 of the control animals the penicillin concentration in the blood was also measured after 60 min infusion to assess whether a steady state had been attained at 90 min.

**Animal preparation**

One day before the experiment, PE 50 polyethylene catheters (Intramedic, Clay Adams, Parsippany, NJ) were inserted into the femoral vein and the tail artery. The catheters were led subcutaneously to a pocket under the skin on the back, filled with heparinized saline, and sealed. Bilateral paracentesis was performed to avoid possible ear pain during the compression. A loose skin suture was tied on each side of the animal to assist in restraining during the experiment. Hypnorm 1 ml/kg i.p. (fluanisone 10 mg and fentanyl 0.315 mg/ml, Mekos, Hälssingsborg, Sweden) was used as anesthetic for these procedures.

Before the start of experiments on the following day, the wound on the back was opened and the catheters were exposed and connected to the infusion pump and the blood sampling system, respectively (12). The catheters were again flushed with heparinized saline, and the animal was restrained in a Plexiglas tube with the previously inserted skin sutures as moorings.

**Compression procedure**

A 4 liter chamber system (12) was used. It was equipped with a high-pressure pump and syringe for infusion of drug, connections and valve for blood sampling, atmospheric and rectal temperature monitoring system, CO\(_2\) and O\(_2\) monitors, a fan for gas mixing, scrubber, and heating systems as described previously (12). After sealing the chamber port, the partial pressure of oxygen was raised to 0.6 ATA, from then on helium was used, with a compression rate of 0.3 ATA/min. The O\(_2\) content was later kept between 0.4 and 0.6 ATA throughout the experiments. The penicillin infusion was started when 71 ATA was reached. The chamber temperature was kept between 32°C and 35°C during the experiments. The CO\(_2\) concentration in the chamber was kept below 0.01 vol/vol% (5.4 Torr).

**Analysis**

The midlobe of the liver, the left kidney, part of the abdominal muscle, perirenal fat, and the right cerebral hemisphere together with the cerebellum were weighed, cut into small pieces, and transferred to glass tubes containing 4 ml Lumasolve (Lumac Systems, Basel, Switzerland)/g brain tissue, the other tissues were mixed with 6 ml Lumasolve/g. The samples were kept for 12 h at room temperature, homogenized using the Potter Elvehjem technique, and sonicated.
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Three milliliters of the homogenates were transferred into counting vials, bleached with 0.3 ml H₂O₂ 30%, and finally 10 ml of a mixture of Lumagel (Lumac Systems) and 0.5 M HCl (9:1) were added. The blood samples were collected into heparinized 1-ml plastic syringes, weighed, and transferred to counting vials. Lumasolve:isopropanol (1:2) 0.5 ml was added, followed by H₂O₂ and Lumagel/HCl as described above. The samples were counted in a Hewlett Packard Model 3375 liquid scintillation spectrometer.

Student's two-tailed t-test was used for statistical analysis.

RESULTS

The observed mean values of 38.4 μg/ml and 37.2 μg/ml at 60 and 90 min, respectively, and the negligible slope of the concentration curve (0.002 μg/ml⁻¹·min⁻¹) suggest that a situation close to steady state was obtained with the present experimental design (Table 1).

The penicillin concentrations in arterial blood were somewhat higher in the pressure experiments (55.0 ± 17.3 μg/ml) compared with 40.8 ± 13.9 μg/ml in the control experiments, but the difference did not reach statistical significance (Table 2). The brain penicillin content was also higher in the pressure group than in the control group (4.0 ± 0.7 vs. 3.3 ± 0.8 μg/g). The values were significantly different at the P < 0.05 level, but due to the higher blood penicillin concentrations in the pressure group, the brain: blood concentration ratio was not significantly different from the controls.

The penicillin concentrations in the liver, kidney, muscle, and fat tissues and the ratios between the tissue and blood concentrations were not significantly different in the pressure group as compared to the control group (Table 2).

DISCUSSION

The present study shows a small increase in the rat brain penicillin content after 90 min constant rate infusion at 71 ATA He-O₂ as compared to 1 ATA air. The mean blood levels of penicillin were also somewhat increased in the pressure experiments, and the brain: blood concentration ratio was therefore not significantly altered. This drug is reported to give a

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>BENZYLPCENICILLIN CONCENTRATIONS* (MEANS ± SD) IN ARTERIAL BLOOD DURING CONSTANT RATE INFUSION (100 mg·kg⁻¹·h⁻¹) IN THE RAT AT 1 ATA WITH CONCENTRATION CURVE SLOPES**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
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<tr>
<td>31.3</td>
<td>34.5</td>
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<td>42.6</td>
<td>42.0</td>
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<td>37.6</td>
<td>36.7</td>
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<tr>
<td>37.5</td>
<td>37.5</td>
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<tr>
<td>35.9</td>
<td>36.6</td>
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<tr>
<td>43.4</td>
<td>40.6</td>
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<tr>
<td>38.1 ± 4.5</td>
<td>38.0</td>
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<tr>
<td></td>
<td>± 2.8</td>
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</table>

*Values in μg·ml⁻¹. **Values in μg·ml⁻¹·min⁻¹.
TABLE 2
Tissue Concentrations of Benzylpenicillin in Rats During Constant Rate Infusion (100 mg·kg⁻¹·h⁻¹) at 1 ATA Air and at 71 ATA He-O₂*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>1 ATA Air</th>
<th>Tissue:Blood Ratio</th>
<th>71 ATA He-O₂</th>
<th>Tissue:Blood Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>40.8 ± 13.9</td>
<td></td>
<td>55.0 ± 17.3</td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>3.3 ± 0.8</td>
<td>0.085 ± 0.029</td>
<td>4.0 ± 0.7**</td>
<td>0.077 ± 0.027</td>
</tr>
<tr>
<td>Liver</td>
<td>119.1 ± 26.1</td>
<td>3.120 ± 1.058</td>
<td>135.5 ± 40.0</td>
<td>2.512 ± 0.940</td>
</tr>
<tr>
<td>Kidney</td>
<td>270.7 ± 95.6</td>
<td>6.914 ± 3.163</td>
<td>222.5 ± 79.9</td>
<td>4.342 ± 2.237</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>14.3 ± 9.6</td>
<td>0.326 ± 0.105</td>
<td>22.8 ± 12.2</td>
<td>0.392 ± 0.183</td>
</tr>
<tr>
<td>Fat</td>
<td>8.2 ± 7.9</td>
<td>0.177 ± 0.093</td>
<td>8.2 ± 7.5</td>
<td>0.120 ± 0.068</td>
</tr>
</tbody>
</table>

*Values are given as means ± SD in micrograms per gram or micrograms per milliliter. 

n = 10 (1 ATA air), n = 11 (71 ATA He-O₂). **P < 0.05.

A brain:plasma ratio of approximately 0.08 (13), which is close to the ratio observed in the present study.

Penicillin is a weak organic acid that is highly ionized at pH 7.4, and in the rat approximately 60% is bound to the plasma proteins. The relative exclusion from the central nervous system is primarily due to these physical properties of penicillin. The permeability characteristics of the brain capillary endothelium constitute another barrier for the distribution of penicillin in its ionized form (14). The slight pH difference between the plasma and the cerebrospinal fluid is too small to affect the distribution of penicillin to any significant extent.

Data on drug distribution at increased pressure are scarce. A recently published study from our laboratory suggests a reduced cerebral uptake of thiopental and diazepam in the rat during the early distribution phase at 71 ATA He-O₂ (8). Studies of the function of the blood:brain barrier in rabbits exposed to 6 ATA air and decompressed, showed that the brain uptake of trypan blue dye (4) and tetracycline (5) were increased after the exposure.

Infusion rate and renal clearance govern the blood level of penicillin during constant rate drug infusion, and 90% of the steady state blood concentration level is reached after approximately 3.3 half-lives (15). In the rat, the penicillin half-life has been reported to be approximately 29 min (16); hence the 90-min duration of the infusion in the present experiments. In our animals, half-life may have been shorter, because the blood levels of penicillin reached a plateau after 60 min. It is also noteworthy that peak brain concentrations of penicillin are reached approximately 15 min after i.v. injections in rabbits (17). Possible blood flow changes in the brain at pressure would not alter the uptake of penicillin which has a diffusion limited passage across the brain capillary membranes (18). On the other hand, flow alterations might influence the glomerular penicillin filtration which accounts for approximately 40% of the penicillin clearance in the rat (19). However, we have recently shown that in the awake rat at 71 ATA He-O₂, the brain as well as total kidney blood flow were unchanged (Aanderud et al. in preparation).

Penicillin is actively excreted from the brain and cerebrospinal fluid to the venous blood by a mechanism common to many weak organic acids including monoamine metabolites (20, 21). The renal penicillin excretion is probably due to a similar mechanism, since the elimination from both organs may be blocked by probenecid (16, 21). One possible interpretation of the increased blood and brain concentrations would be that the active excretory processes both in the central nervous system and the kidneys are inhibited by the hyperbaric environment.
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However, studies of the uptake of organic ions by rat renal cortex slices at 30.6 ATA do not indicate gross impairment of the transport of organic acids and bases (22).

In human subjects, increased diuresis has been reported during the first phase of deep simulated dives (23), and in rats at 21 ATA He-O₂, the antidiuretic effect of morphine is decreased (24). It is therefore conceivable that increased diuresis may decrease extracellular body water and thereby the distribution volume of penicillin, contributing to the slightly increased blood concentrations of the drug at pressure.

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Aanderud L, Bakke OM. Distribution tissulaire de la pénicilline chez des rats durant une infusion à vitesse constante à 71 ATA. Undersea Biomed Res 1985; 12(1):53–58.—De la benzyl [¹⁴C] pénicilline fut infusée chez des rats pendant 90 min à une vitesse constante de 100 mg · kg⁻¹ · h⁻¹ à 1 ATA d’air, le groupe témoin (n = 11) et à 71 ATA d’hélio, le groupe pression (n = 11). La radioactivité fut mesurée dans le sang artériel, ainsi que le cerveau, foi, rein, muscle et tissus adipeux. La concentration moyenne de pénicilline dans le cerveau après 90 min d’infusion était augmentée d’environ 20% à 71 ATA (P < 0.05), mais le rapport entre les concentrations cérébraux: sang ne fut pas affecté de façon significative. Le contenu moyen en pénicilline du sang, foie, et muscle était légèrement plus élevé et le contenu moyen du rein était un peu plus bas sous pression, mais aucun changement statistiquement significatif ne fut observé.

pénicilline distribution de la drogue
hélium haute pression

REFERENCES