Analysis of sulfamethazine fates: an animal experimental with limited N-acetyltransferase as a models

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ABSTRACT

Sulfamethazine has been reported to destroy in vivo infection caused by positives and negative gram microorganism. The objective of this study was to analyze the absorption, distribution, metabolism and excretion of sulfamethazine in subjects with limited N-acetyltransferase (mixed breed species and original breed species). We determined the kinetics parameter of ten mixed breed subjects and ten original breed subjects. The mixed breed subjects were used Bali mixed breed dogs and the original breed subjects were used German shepherd dogs. The absorption, distribution, metabolism and excretion kinetics and metabolic phenotypes of subjects with mixed breed condition were determined by spectrophotometer UV-Vis at 545 nm wave length and compared with original breed dogs. The result research showed that several of kinetic parameters between two species of breed dog were similar at p>0.05. The incidence of the poor metabolizer genotype between two species of dog was similar (p>0.05). The absorption, distribution, metabolism and excretion profiles of sulfamethazine between two species dog of limited N-acetyltransferase (original dog and mixed breed) were similar.

Keywords: Sulfamethazine, absorption, distribution, metabolism, excretion, dog

Analisis nasib sulfametazin : suatu percobaan pada hewan dengan keterbatasan N-asetiltransferase sebagai model

ABSTRAK

Sulfametazin sudah dilaporkan mampu membasmi infeksi yang disebabkan oleh mikroorganisme gram positif dan negatif secara in vivo. Studi ini bertujuan untuk melakukan analisis tentang absorpsi, distribusi, metabolisme dan eksksresi dari sulfametazin pada subyek dengan keterbatasan N-asitiltransferase (species turunan campuran dan spesies turunan murni). Pada penelitian ini ditetapkan parameter kinetik dari absorpsi, distribusi, metabolisme dan eksksresi pada sepuluh subjek turunan campuran dan sepuluh subjek turunan murni. Subjek turunan campuran yang digunakan adalah turunan galur campuran anjing Bali dan turunan murni yang digunakan adalah galur anjing gembala Jerman. Kinetik absorpsi, distribusi, metabolisme dan eksksresi serta metabolik fenotipe pada subjek turunan campuran ditetapkan menggunakan spektrofotometer lembayung ultra-tampak pada panjang gelombang 545 nm dan diperbandingkan dengan anjing gembala Jerman. Hasil penelitian menunjukkan variasi parameter kinetik antara dua subjek anjing turunan tersebut, tidak berbeda nyata pada p>0.05. Kejadian keterbatasan aktifitas metabolisme secara genotipe diantara dua subjek anjing turunan tersebut bersifat sama (p>0.05). Absorpsi, distribusi, metabolisme dan eksksresi dari sulfametazin pada kedua spesies anjing (turunan galur campuran dan murni) dengan keterbatasan N-asitiltransferase tidak berbeda.

Kata kunci: Sulfametazin, absorpsi, distribusi, metabolisme, eksksresi, anjing
INTRODUCTION

Sulfamethazine has been reported to destroy the diplococci or gram positive cocci and bacilli or gram negative diplococci and to reduce the in vivo infection caused by other gram positives microorganism. The rate of sulfamethazine in American black were fastest then Caucasians. These phenomenon showed that the expression of N-acetyltransferase (NAT-2) as a metabolism enzyme for each genetic population was different. The rate of disappearance of sulfonamides groups from plasma shows a bimodal distribution, allowing the identification of individuals as rapid or slow acetylators (inactivates of the drug). The Caucasians were apparently homozygous recessive gene of N-acetyltransferase expression with limited metabolism activity. In other subject of limited N-acetyltransferase with original breed genotype like rhesus monkey, the sulfonamides groups showed slow metabolism. If the original genotyping subject were to be married with other mixed breed, the new generation will have polymorphism pattern. In dog species, some references described that species did not have NAT-2 in their liver for metabolism of N-acetyl compounds. But other researcher explained dogs have limited NAT-2 in their microsomal liver and appeared at 6-month of age. As reported by Sallovitz et al., dog population have N-acetyltransferase at hepatic microsomal at about 2 to 3% from all enzyme on their bodies. If the original breed dog to be married with mixed breed dog, their children may have a varieties of profiles of metabolism activities at uniformity or polymorphism. In this study, the fates of sulfamethazine in two different species of limited N-acetyltransferase subjects following extra vascular route were analyzed. As a subject model dogs was used with assumption that their metabolism activities was similar to human with limited N-acetyltransferase like a population of Afro-Amercia.

The objectives of this study was to investigate the differences of absorption, distribution, metabolism and excretion (ADME) and metabolite product between original breed species and mixed breed species of limited N-acetyltransferase. The outcome of this research suggested that it can be used for designing model of regimened dosage form of drug for use in clinical case in human with limited-acetyltransferase.

METHODS

Research design, Chemical and Instrumentation

A time series design was conducted with sulfamethazine and breed subject as an independent variable and kinetic parameters of ADME as a dependent variable. Sulfamethazine as certified reference material (CRM) was obtained from WHO Collaborating Center for Chemical Reference Substances Apoteksbolaget AB, Centrallaboratoriet S-105 14 Stockholm Sweden. The drug (pharmaceutics grade) was obtained from Kimia Farma drug store, Surabaya. Artificial plasma from subject with limited N-acetyltransferase was obtained from The Center of Veterinaria Farma, Surabaya. Trichloro acetic acid, NaNO3, Amonium sulphamate, NaOH, HCL, N-(1-Naphthyl)-ethylenediamin (NED) were obtained from Merck Corp., p.a grade. For the instrument of analysis Spectrophotometer UV-Vis Hitachi 1100 was used.

Research subjects and drug administration

The research subjects included two species of dogs (original breed dogs or German shepherd dog and mixed breed dog or Bali breed dogs) from Surabaya population. The twenty male, adult and healthy subjects weighed between 12
to 17 kg used as a co-morbid criterion for these 
researches. The animals were maintained at animal 
welfare grade. All of the subjects were examined 
for serum glutamic oxaloacetic transaminase 
(SGOT), serum glutamic pyruvic transaminase 
(SGPT), creatinine, and blood urea nitrogen 
(BUN) levels before the trials. The animals were 
acclimatized for 3 week prior to commencement 
of the experiments. They were separated into two 
separate groups of ten breed dog each population 
and given 50 mg.kg\(^{-1}\) sulfamethazine by deep intra 
muscular route at gluteus medialis.

**Measured of linearity, precision and accuracy**

Vxo was used for measurement of linearity 
as a standard curve series in distilled water at 
series 0, 25, 50, 100, 150, 200, 300, 400 and 500 
µg.ml\(^{-1}\) and in artificial plasma at series 50, 100, 
200, 300, 400, 500 µg.ml\(^{-1}\). For measured 
precision and accuracy in artificial plasma of the 
analytical method were used coefficient of 
variation in percent (%CV) and percent recovery 
from ratio sulfamethazine in distilled water to 
artificial plasma at 50, 100, 200, 300, 400 and 
500 µg/ml.\(^{(10)}\)

**Blood sampling**

Blood samples (5 ml) were collected from 
saphen vein in heparinized glass centrifuge tubes 
at 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 
330, 390, 450, 510 minutes after the 
administration of drug. The plasma was separated 
after centrifugation at 3000 rpm for 10 minutes 
and store at –20 °C.

**Samples pretreatment**

All samples were thawed from deep freezer, 
and 0.1 ml of blood plasma and was put in to 
centrifuge test tube 10 ml. Added 3.9 ml aqua pro 
 injection free from O2 and shake up well for 10 
minutes. The samples were added with 1 ml (2%) 
of trichloro acetic acid, shake up well for 10 
minutes and centrifuged at 3000 rpm for 10 
minutes. The process separated in two serial 
sample preparations.

**Samples preparation for obtained free 
sulfamethazine**

From samples pretreatment, 2 ml of 
supernatant was taken, added the supernatant 
with 0.2 ml of HCl (shake up well at 10 
minutes) and kept the serial test tube in cold 
room (22-25 °C) at about 10 minutes. Added 
0.1 ml of NaNO3 0.1% and shakes up well 
for 10 minutes. Added 0.2 ml of 0.5% of 
ammonium sulphamat. The serial test tubes 
were protected from the light using carbon 
paper. Added the solutions with 0.2 ml NED 
0.15% and shakes up well for 10 minutes. The 
tubes were added with 0.2 ml of HCl and 
shakes up well at 10 minutes. Added with 3.8 
ml of aqua free from O2 and measured free 
sulfamethazine by spectrophotometer at 
maximum wavelength.

**Samples preparation for obtained total 
sulfamethazine**

From sample pretreatment at sampling 
time 330, 390, 450 and 510 minutes, put the 
supernatant 2 ml, added 0.2 ml of HCl and 
shakes up well for 10 minutes. All samples 
were marked to the borderline of meniscus with 
sign pen. Serial test tube was closed with watch 
glass and pressed the watch glass with glass 
ball. All the samples were hydrolyzed at 100 
°C (30 minutes) in the water bath. Added with 
aqua free O2 up to meniscus border sign. 
Added 0.1 ml NaNO3, and shakes up well for 
10 minutes. Added with 0.2 ml of ammonium 
sulphamate 0.5%, and shakes up well for 10 
minutes. The serial test tubes were protected 
from the light by covered carbon paper. Added 
with 0.2 ml NED 0.15%, and shakes up well 
for 10 minutes. Added 3.8 ml aqua free from 
O2 and measured the concentration by 
spectrophotometer at maximum wavelength.
Data analysis

Analyses of linearity, precision, accuracy used PV software program. (11-13) Fates of sulfamethazine were analyzed by pharmacokinetic parameters with Topvit 2.0 pharmacokinetic software program. (3) Availability criterion of sulfamethazine by intra muscular administrated used absorption assumption at 100% (F=1). The characterization kinetic parameters of mixed breed species were compared with kinetic parameter from original breed by Minitab 13.0 software program with independent t test at significant 0.05. The metabolite product of sulfamethazine between mixed breed and original breed subject were analyzed by metabolite product ratio as described in equation 1 below. For characterization of fastest, slowest or moderate phenotypes slope (b) from linear regression relationship (Y = a + bX) of metabolite product ratio (Y) vs. times (X) at 4th data sampling from the last were analyzed. Each slope from two species will be analyzed by mode test for phenotype metabolite product characterization between two breed species.

Equation 1

\[
\text{Metabolite product ratio} = \frac{\text{Metabolite product concentration}}{\text{Total drug concentration}} \times 100\%
\]

RESULT

The result of SGPT, SGOT, creatinine and BUN examined for all subject were normal, with a range of 8.0-11.2 U/L for SGPT, 36.0-39.0 U/L for SGOT, 0.43-0.70 mg.dl\(^{-1}\) for creatinine and 8.0-12.0 mg.dl\(^{-1}\) for BUN. The dogs appeared to be homogenous as research subjects and ready for use for treatment of absorbable sulfonamide groups. The concentration were 0, 25, 50, 75, 100, 150, 200, 300, 400, 500 µg.ml\(^{-1}\) in distillated water; the respond of sulfamethazine was linear (r = 0.99), with value of coefficient variation from function (Vx0) 1.25%. The concentration range 50, 100, 200, 300, 400, 500 µg.ml\(^{-1}\) in triplicate artificial plasma, the average respond of this drug was linear (r = 0.99), with values of coefficient variation from function (Vx0) 3.48% to 6.06%. Mean coefficient of variation (CV) within days were 2.60% (for 500 µg.ml\(^{-1}\)) to 10.91% (for 50 µg.ml\(^{-1}\)), but % recovery was between 89.09% (50 µg.ml\(^{-1}\)) to 97.31% (500 µg.ml\(^{-1}\)). The precision between samples in artificial plasma was actually quite good (CV<20%). We believe that this is an accurate assay with great reproducibility. (13) The pharmacokinetic parameters between two species breed dogs were described below (Table 1 and Figure 1).

Table 1. Kinetics parameter of sulphamehazine in mixed breed (Bali breed) and original breed (German shepherd) after treatment of sulphamethazine 50 mg.kg

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Mixed breed (Bali breed) ((n=10))</th>
<th>Original breed (German shepherd) ((n=10))</th>
<th>Significance level</th>
</tr>
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<tbody>
<tr>
<td>(T_{\alpha} (\text{Hour}))</td>
<td>12.62 ± 5.81</td>
<td>17.22 ± 9.10</td>
<td>NS</td>
</tr>
<tr>
<td>(T_{\text{max}} (\text{Hour}))</td>
<td>4.21 ± 0.96</td>
<td>5.13 ± 1.12</td>
<td>NS</td>
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<tr>
<td>(C_{\text{max}} (\mu g.ml^{-1}))</td>
<td>72.18 ± 20.45</td>
<td>65.64 ± 24.85</td>
<td>NS</td>
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<tr>
<td>(Vd (L.Kg^{-1}))</td>
<td>8.15 ± 2.22</td>
<td>22.61 ± 23.34</td>
<td>NS</td>
</tr>
<tr>
<td>(Cl (mL.min^{-1}))</td>
<td>8.38 ± 5.39</td>
<td>8.56 ± 5.63</td>
<td>NS</td>
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</table>

Values are Mean ± SD
NS = P>0.05, significantly not different from the value obtained with the vehicle by student’s t test
\(T_{\alpha}=\) elimination half-life \(T_{\text{max}}=\) elimination maximum \(C=\) clearance
\(Vd=\) volume distribution \(Cl=\) total clearance
The time sampling versus plasma concentration presented in Figure 1 for mixed breed species (a) and original breed species (b) were apparently different from each other. That result showed the sampling times of individual mixed breed dogs were not exactly 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 390, 450, 510 minutes. That because, the individual behavior of mixed breed dogs not easy to handle. In contrast, the original breed dogs were very easy to handle and gave exact time sampling of 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 390, 450, 510 minutes. But the differences of time sampling at 30 to 510 minutes between the two species did not affect result analysis of plasma concentrations.

Result analyses of metabolite product phenotypes between two breed species were illustrated in Table 2a,b and Table 3 below. Table 2a and 2b showed different time sampling. This was caused by the individual mixed breed dogs were not easy for blood sampling. The characterization slope of metabolite product gave one mode (moderate phenotypes) profile for all subjects as illustrated at Table 3 and histogram Figure 3 the below.

**DISCUSSION**

In general, parameter kinetics between two species breed of limited N-acetyl transferase were similar as described in Table 1. The availability of sulfamethazine between two breed species was similar as illustrated by the mean values of clearance total. Availability of sulfamethazine in two species of dog can be measured from rate of elimination to clearance (C = rate elimination/clearance). The rate of elimination (β) was measured from analysis of 0.693/T½β. The elimination half-live (T½β) between two species of dog was similar as illustrated at Table 1 with p>0.05.

<table>
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<tr>
<th>Time (min)</th>
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<th>n-2</th>
<th>n-3</th>
<th>n-4</th>
<th>n-5</th>
<th>n-6</th>
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Table 2a. Metabolite product of sulfamethazine in original breed species (German shepherd)
Table 2b. Metabolite product of sulfamethazine in mixed breed species (Bali breed)

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<tr>
<th>Time (min)</th>
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<td>25.279</td>
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Slope: 0.154 0.091 0.017 0.163 0.091 0.183 0.189 0.202 0.184 0.170

- : Failure

Table 1 showed differences of kinetic parameter values between two species of breed dog. Statistically the two groups were similar. That is because some individuals in the two groups of dogs had similar kinetics parameter values as described by Lazuardi.\(^{(14)}\)

This will be specially important for a drug with narrow therapeutics index on subject with limited n-acetyltransferase where there was only a small difference between the concentration producing therapeutic benefit and the concentration that will produce toxic manifestations. The sulfamethazine in subject with limited N-acetyltransferase was illustrated at Figure 1. Figure 1a and 1b showed the disappearance of sulfamethazine from plasma in mixed breed species and original species were gradual. Phenomenon of the sulfamethazine in mixed and original species showed limited metabolism. As illustrated in Table 2a and 2b, the influence of different breed on metabolism activities of sulfamethazine in dog species were smallest.

Table 3 and Figure 2 showed uniform profile at mode values of 0.068 (Table 3). The histogram of Figure 2 at uniform mode was apparently normal distribution as illustrated in Figure 2 with red lines.

Tanchev et al.,\(^{(3)}\) mentioned the influence of different factors (breed, species, gender) on fates of drug in animals or human subjects. Some species may be significantly different but other species may be similar. The diminazene aceturate and suramin for examples with bi mode profile on goat and poly mode on rat species.\(^{(15,16)}\)
Available of sulfamethazine in human with limited N-acetyltransferase perhaps similar with the dog as an animal model. In some population, Caucasians and Mongoloid were different to Negroid as human with limited N-acetyltransferase. Some researcher described that Negroid population was sensitive to sulfamethazine with accumulated risk.(17)

CONCLUSION

The ADME profiles of sulfamethazine between two species of dog of limited N-acetyltransferase (original dog and mixed breed) were similar at p>0.05 with modus value at 0.068. We must be careful when giving absorbable sulfonamides group on subject with limited N-acetyltransferase although at usual dose.

ACKNOWLEDGEMENT

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Table 3. The slope analysis of metabolite product

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<td>1st</td>
<td>0.017-0.034</td>
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</tr>
<tr>
<td>2nd</td>
<td>0.053-0.092</td>
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<tr>
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<td>5th</td>
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</table>

Class average = 0.202-0.017 = 0.185
Minimum class number = 1 + (3.3) log n = 5.2 (or 5)
Class interval (p) = 0.185/5 = 0.037
b = 0.0545; b1= 6-3 = 3, b2 = 6-1 = 5
Mode = b + p {b1: (b1+b2)} = 0.068

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