Increased Susceptibility of *S. aureus* to Ampicillin during Exposure to Rifampicin : A Distinct Mechanism of Antimicrobial Synergism

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Abstrak

Efek sinergistik rifampisin dan ampisilin telah diteliti pada 20 isolat S. aureus. Isolat yang dipakai adalah yang resisten terhadap ampisilin dan sensitif terhadap rifampisin. Kepekaan isolat terhadap rifampisin ditentukan dengan cara dilusi cair dan resistensi ampisilin ditetapkan dengan cara difusi cakram menurut Kirby-Bauer. Uji iodometrik dilakukan untuk mengetahui produksi β-laktamase oleh bakteri. Perubahan kepekaan S. aureus terhadap ampisilin dinilai dengan menentukan Kadar Hambat Minimal (KHM). Hasil menunjukkan bahwa sebagian besar isolat yang resisten terhadap ampisilin dengan KHM lebih dari 5 µg/ml berubah menjadi sensitif dengan KHM lebih kecil dari 0,25 µg/ml. Perubahan KHM hanya terjadi pada isolate yang memproduksi β-laktamase. Disimpulkan bahwa terdapat efek sinergistik rifampisin dan ampisilin terhadap S. aureus penghasil β-laktamase yang kemungkinan besar berdasarkan hambatan sintesis β-laktamase oleh rifampisin.

Abstract

Synergistic affect of rifampisin and ampicillin has been studied on 20 isolates of S. aureus. Isolates used were ampicillin resistent and rifampicin sensitive. Susceptibility of the isolate to rifampicin was determined with standard broth dilution method and resistance to ampicillin was ascertained by disk diffusion method of Kirby-Bauer. Additional simple iodometric test was performed to detect β-lactamase production of the bacteria. Alteration in susceptibility of S. aureus to ampicillin was evaluated by determining Minimal Inhibitory Concentration (MIC) of this agent without and with exposure to rifampicin at sub MIC. Results show that most ampicillin resistant isolates with MIC of > 5 µg/ml turn to sensitive ones with MIC value of less than 0,25 µg/ml. The change of MIC value were observed only in β-lactamase positive isolates. We concluded that there is synergistic effect of rifampicin and ampicilin which most likely mediated by inhibition of β-lactamase production by rifampicin.

Keywords : *S. aureus, ampicillin, rifampicin, synergism*

Bacterial resistance to antibiotics is still a continuing problem worldwide. Every discovery and clinical application of new antimicrobial is always followed by the emergence of bacterial strains resistant to the agent in question within relatively short period of time. Proportion of these resistant strains is increasing time after time.

It was reported in 1991 that 60% of isolates of S. aureus in Surabaya were resistant to ampicillin and this proportion reached 94% by 1993.[1,2] The same fenomenon also occured in Yogyakarta as reported by Trihendrokesowo and Hera Nirwati.[3,4]

In case of *S. aureus,* resistance to ampicillin is usually mediated by plasmid conferring gene for β-lactamase production.[5]

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Despite the quest for new antimicrobials, in order to overcome the problem, several strategies have been employed. The use of antimicrobial combination such as penicillin and gentamicin is widely acceptable, especially in serious life-threatening infections. Combination of trimethoprim and sulfamethoxazole is directed to block folate metabolic pathway of bacteria at two different sites. More recently, enzyme inhibitors have been incorporated to antibiotics in order to block the enzyme produced by bacteria which can destroy the antibiotics.

In principle, combination of antimicrobials is aimed at disturbing resistance mechanism of the bacteria. Specifically, the combination is indicated for treatment of mixed bacterial infection, enhancement of antibacterial activity, and prevention of emergence of resistant strains.[6]

Rifampicin is an antibiotic which is used especially for the treatment of tuberculosis. This agent inhibits DNA-dependent RNA polymerase of the bacteria so that initiation of transcription process is blocked.

In addition to M. tuberculosis, rifampicin is also active against Gram positive and Gram negative bacteria. Sensitive strains will be killed by less than 1 μg/ml.[6,7]

In this study we search for possible synergistic effect or rifampicin and ampicillin against *S. aureus*. The rationale behind the study is that rifampicin inhibits transcription of β-lactamase gene so that ampicillin will not be destroyed. The intact ampicillin will then exert its action against *S. aureus*.

METHOD

Isolates from clinical specimens identified as *S. aureus* were collected. Susceptibility of each isolate to ampicillin was determined by disk diffusion method of Kirby-Bauer, and simple iodometric test was performed to detect β-lactamase produced by the isolates.[8] Minimal Inhibitory Concentration (MIC) of rifampicin against each isolate of *S. aureus* were determined by standard broth dilution method.[9]

Since MIC breakpoint of rifampicin for susceptible isolates is ≤ 1 μg ml, the isolates showing MIC of more than 1 μg/ml were then excluded from further study. Determination of MIC of ampicillin without addition of rifampicin was performed as control. At the same time, MIC of ampicillin with addition of rifampicin was determined for each isolate. Rifampicin was added at dose equivalent to half MIC of each isolate. Addition of rifampicin at sub MIC (without ampicillin) was also included as control in each assay. MIC value was read after overnight (18-24 h) incubation at 37°C. Exclusion of rifampicin resistant isolate (MIC > 1 μg/ml) is based on the fact that the resistance is due to mutation of RNA polimerase. Mutated RNA polimerase is not affected by rifampicin.[10]

RESULTS

All 22 isolates of *S. aureus* were resistant to ampicillin. From determination of MIC of rifampicin we found that 2 isolates were resistant to rifampicin (MIC value of more than 1 μg/ml) (Table 1).

MIC of ampicillin without and with addition of rifampicin is presented on table 2. Without exposure to rifampicin, all isolates showed an MIC of > 5 μg/ml. This MIC reduced to a value of < 0,2 μg/ml in all, except two, of isolates.

Table 1. Minimal Inhibitory Concentration (MIC) of rifampicin against isolates of *S. aureus*

No.	Isolates	MIC (μg/ml)
1.	SA 1438	0,3125
2.	SA 1409	0,0625
3.	SA 1459	0,0312
4.	SA 1470	0,0156
5.	SA 1512	0,0078
6.	SA 1521	0,0078
7.	SA 1546	0,0078
8.	SA 1560	0,0039
9.	SA 1634	0,0156
10.	SA 1608	> 2
11.	SA 1654	0,0156
12.	SA 1658	0,0312
13.	SA 1665	0,0312
14.	SA 1697	0,06
15.	SA 1597	0,25
16.	SA 1737	0,25
17.	SA 1775	0,015
18.	SA 1778	0,032
19.	SA 1788	0,125
20.	SA 1800	0,03
21.	SA 1801	> 2
22.	SA 1807	0,015

Table 2. MIC of ampicillin for *S. aureus* without and during exposure to rifampicin at sub MIC and its relation to β-lactamase production

No.	Isolates	MIC of ampicillin (μg/ml)		β-lactamase production
		Without exposure	During exposure	
1.	SA 1438	> 5	0,03	+
2.	SA 1409	> 5	0,06	+
3.	SA 1459	> 5	0,03	+
4.	SA 1470	> 5	0,03	+
5.	SA 1512	> 5	0,15	+
6.	SA 1521	> 5	0,15	+
7.	SA 1546	> 5	1,25	+
8.	SA 1560	> 5	> 5	-
9.	SA 1634	> 5	0,15	+
10.	SA 1654	> 5	0,15	+
11.	SA 1658	> 5	0,075	+
12.	SA 1665	> 5	0,15	+
13.	SA 1697	> 5	0,15	+
14.	SA 1597	> 5	0,15	+
15.	SA 1737	> 5	0,15	+
16.	SA 1775	> 5	0,15	+
17.	SA 1778	> 5	0,075	+
18.	SA 1788	> 5	0,15	+
19.	SA 1800	> 5	0,075	+
20.	SA 1807	> 5	0,15	+

DISCUSSION

In this study we found that all 22 isolates of *S. aureus* were resistant to ampicillin. It is not surprising since these isolates usually come from patients who have received antibiotic, especially β-lactam antibiotic, before the doctor sent their specimens to the laboratory for isolation and antibiotics susceptibility test of causative bacteria.

Twenty out of 22 isolates were susceptible to rifampicin as shown by their MIC value (Table 1). Sensitive isolates were inhibited by ≤ 1 μg/ml of rifampicin. MIC of ampicillin for all isolates was greater than 5 μg/ml, and since there is no MIC breakpoint of ampicillin for resistant isolate, this MIC could be much higher. For sensitive isolates, MIC breakpoint has been determined to be ≤ 0,25 μg/ml.[9]

During exposure to rifampicin at sub MIC, we observed that MIC of ampicillin for most isolates was considerably reduced. The decrease of MIC was so great that resistant isolates turn out to be sensitive (Table 2).

Ampicillin resistance mechanism of *S. aureus* includes failure of the agent to enter bacterial cytoplasm because of alteration of cell wall, inability of the agent to bind to the target, and inactivation of the agent by β-lactamase.[10] Iodometric test in this study showed that ampicillin resistance in most isolates is mediated by β-lactamase production. One isolate (SA 1560) did not produce β-lactamase and MIC of ampicillin for this isolate was not changed during exposure to rifampicin. Resistance mechanism of this *S. aureus* might be mutation of either cell wall porin or target site (Penicillin Binding Protein).

Exposure of *S. aureus* to rifampicin at sub MIC does not kill the bacteria. Nevertheless, there should be reduction of gene expression due to partial inhibition of RNA polimerase by rifampicin. Logically, expression of all genes will be inhibited and this alteration is quantitative in term that only the amount of gene product is reduced. Mutant product due to mutation on the gene can not be changed into "normal" product with exposure to rifampicin. From this point of view and based on our data, it is shown that only β-lactamase-producing *S. aureus* that have their ampicillin susceptibility altered by rifampicin.

Although rifampicin alone can be used to kill *S. aureus*, combination of rifampicin and ampicillin will be more advantageous because lower dose of rifampicin is used.

As mentioned before, rifampicin principally inhibits protein or enzyme synthesis by blocking transcription process. From this point we suggest that all antimicrobial resistance mediated by production of bacterial enzyme could be disturbed by rifampicin, this would include resistance phenomen of bacteria other than *S. aureus*.

So far, rifampicin is used exclusively for the treatment of mycobacterial infection. The use of this agent for other infections is uncommon. However, based on our data, combination of rifampicin and ampicillin may be useful in the treatment of selected cases of infection where other antimicrobials are not applicable.

REFERENCES

1. Soeharto S, Harsono S, Mudihardi E. Pola Kepekaan Kuman Terhadap Siprofloksasin. Mikrobiologi Klinik Indonesia 1991; 5 (2): 137- 140.
2. Soeharto S. Studi Bakteriologik Enzim β-laktamase Pada Beberapa Jenis Kuman Serta Kepekaannya Terhadap Sefuroksim. Mikrobiologi Klinik Indonesia 1993; 6 (2): 4-7.
3. Trihendrokesowo, Tjarkiah Appandi, Ristanto, Susi Iravati, Ning Rintiswati, Amin Romas. Macam Kuman dan Pola Kepekaannya terhadap Beberapa Antibiotika. Mikrobiologi Klinik Indonesia 1987; 2 (1) : 6-12.
4. Hera Nirwati. Produksi β-laktamase pada *S. aureus* dan *E. coli* Beserta Pola Kepekaannya Terhadap Beberapa Antibiotika (Scientific Report), Yogyakarta. Fakultas Kedokteran Universitas Gadjah Mada, 1992.
5. Hardy K. Bacterial Plasmids, Berkshire Van Nostrand Reinhold Co. Ltd. 1986.
6. Gilman AG, Goodman LS, Rall TW, Murad F. Pharmacological Basis of Therapeutics. New York. Mac Millan Publishing Co. 1992.
7. Jawetz E, Melnick JL, Adelberg EA, Brooks GF, Butel JS, Nicholas Ornston L. Medical Microbiology 20th ed. London. Lange Medical Book. Prentice-Hall International Ltd. 1995.
8. Lennette EH, Balows A, Hausler WJ, Truant JP. Manual of Clinical Microbiology 3rd ed. Washington : American Society for Microbiology. 1980.
9. Balows A, Hausler WJ, Hermann KL, Isenberg HD, Shadomy HJ. Manual of Clinical Microbiology 5th ed. Washington: American Society for Microbiology, 1991.
10. Greenwood D and O'Grady F. The Scientific Basis of Antimicrobial Chemotherapy. Cambridge. Cambridge University Press. 1985.