

Group A2 Analogs of Innovator Teicoplanin: Variability of their Ratio during Treatment Time Course in Human Blood; Consequences for the Generics

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Abstract

Absence of therapeutic equivalence (TE) of generic antibiotics versus the innovator has been reported, even in case of pharmaceutical equivalence (PE). The pharmaceutically active principle of teicoplanin is mainly represented by the A2 group analogs (A2-1 to A2-2) which exist in given ratios in the innovator.

We studied the evolution of these ratios in humans volunteers receiving 2 dosage regimen (6mg/kg IV every 12 hours, 3 times, and then 6 mg/kg per day for ten days - group 1-, and 12 mg/kg IV every 12h, 3 times, followed by 15 mg/kg every other day till D11 - group 2-), by a specific HPLC methodology, after single dose (D1) and at steady state at day 11(D11).

The less lipophilic analogs A2-1, A2-2, and A2-3 percentage decreases respectively were 18%, 11%, and 21% (group 1) and 34%, 14%, and 15% (group 2) between D1 and D11 for trough concentrations. At the same time, the more lipophilic A2-4 and A2-5 analogs ratio increases respectively were 15% and 19% (group 1) and 13% and 30% (group 2). These variations raise the problem of generics having the same global qualitative composition of analogs (PE) but with differing initial ratios. The bactericidal activity of innovator teicoplanin is optimal at its own ratios of the different analogs that act in a synergistic manner. Regarding generics, it would lead to final ratio in blood and tissues that are at risk of a sub optimal bactericidal activity. Thus, concluding in bioequivalence and therapeutic equivalence of these generics with the innovator remains highly questionable.

Keywords: Teicoplanin; A2 analogs; innovator; generics; therapeutic equivalence

Introduction

An increasing number of recently published papers report the absence of Therapeutic Equivalence (TE) of generic antibiotics when compared with the innovator, even in case of Pharmaceutical Equivalence (PE). On the basis of *in vitro* PK/PD data or *in vivo* experimental infections data, generics of antibiotics such as gentamicin, oxacillin, vancomycin and more recently teicoplanin have been reported to present risks of failure of therapeutic equivalence although PE was proven [1-5]. Indeed, according to the official WHO definition [6], an antibiotic drug, to be considered as a generic, must, at first, exhibit the same qualitative and quantitative composition of active substance than the innovator. Then the bioequivalence must be proven by either an equivalent bioavailability or equivalent therapeutic effect between the generic and the innovator. This is also stated by the FDA and EMEA [7], for who TE results from PE plus bioequivalence. By definition, for intravenous drugs, the bioequivalence is always reached as far as the bioavailability is considered to be 100% as no absorption or resorption processes are involved. Nevertheless, one has to keep in mind that, even with IV drugs, a 100% bioavailability does not necessarily imply bioequivalence: a short duration IV bolus may result in the same area under the curve (AUC) than a 1h infusion of the same amount, but accompanied with a higher peak concentration, what may result in a different efficacy for concentration- dependant antibiotics. Currently, because two IV forms are considered bioequivalent, the assessment of TE of generics when compared with innovator only depends of the quality of the raw materials used in the generic [6,7].

Teicoplanin is an injectable antibiotic produced by fermentation of *Actinoplanes teichomyceticus*. The pharmaceutically active principle is teicoplanin A2, a complex mixture of compounds designated A2-1 to A2-5, all differing in their length and branching of the fatty acid moiety

linked to the glucosamine residue. They account for 90-95% of the total teicoplanin complex. A sixth more polar active component, teicoplanin A3, is always present in the final product, but does not exist in the fermentation broth. It has been identified as a degradation product of teicoplanin A2. It has been recently shown [8] that the production of the individual factors A2-2 to A 2-5 can be deeply influenced by the composition of the fermentation medium. The addition in the medium of precursors such as methyl linoleate, trilinoleate and crude cottonseed and/or corn oils can enhance the production of A2-1. In the same way, addition of methyl oleate, trioleate and olive and/or lard oils can stimulate the production of A2-3. The percentage of A2-2, A2-4 and A2-5 are significantly increased by the addition of amino acids such as L-leucine, L-isoleucine and L-valine. It results that the final composition of teicoplanin in terms of the proportion of each component is deeply influenced by the fermentation medium composition and the production process. It is thus logical to consider that, depending on the manufacturing process; the five major components of teicoplanin may have different quantitative distribution in the current generics. The European Pharmacopoeia monograph for teicoplanin [9] gives

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the following limits for the acceptability of the different components ratio: 35-55% for A2-2, and an undifferentiated upper limit of no more than 20% for the other components. Colombo et al. [10] compared the composition of eleven batches of the innovator (Targocid/Targosid) with fourteen generics from different manufacturer. They concluded to statistically significant differences for Teicoplanin A2-1 (A2-1), A2-2, A2-3, A2-4 and A2-5 between some of the generics and the innovators. The distribution of the components of the generics unpredictably changed with the manufacturer. This is a concern for patients: Hanada et al. [11] showed that the six analogs of teicoplanin have different activities against specific bacterial species, and due to their different chemical structure, the different analogs have variable lipophilicity resulting in variable protein binding and pharmacokinetic behavior. Bernaregi et al. [12] concluded to a correlation between lipophilicity and decrease of some PK parameters such as total and renal clearances. They predicted a more pronounced accumulation of the more lipophilic compounds A2-4 and TA2-5 versus the others.

In a study published a few years ago [13], we investigated the trough and peaks concentrations of teicoplanin in volunteers at single dose and at steady state, after the administration of two dosage regimen: 6 mg/kg/24 h and 15 mg/kg/48 h. Only the total teicoplanin concentrations measured by fluorescence polarization immunoassay (FPIA) were published and used for PK parameters calculation. However, all the serum concentrations for the whole study were also measured by high performance liquid chromatography which allowed the measurement of all the A2 group analogs individually (A2-1 to A2-5).

We present here the results of the evolution, during this study, of the five compounds and their respective ratio at trough and peak concentrations at day 1 (single dose) and day 11 (steady state).

Study Subjects and Procedure

The study was a single-center, randomized, open-label, parallel group, control trial including 24 volunteers [13]. Two treatment groups were developed. Group 1 received 6 mg/kg intravenously every 12 hours 3 times and then 6 mg/kg per day from day 3 to 10, and group 2 received 12 mg/kg intravenously every 12 hours three times and then 15 mg/kg every other day on days 3, 5, 7 and 9.

Teicoplanin Dosing and Sampling Times

Four hundred mg freeze dried teicoplanin-containing vials (batch A0007; Gruppo Lepetit, Anagni, Italy) with a 24-mg sodium chloride excipient that had been stored at room temperature for a maximum of 3 months were reconstituted with 3.2 ml of sterile water just before each infusion. A strictly weight-adjusted dose was given by a direct manual 1-min infusion in the ante-cubital vein.

The concentrations in the sera of subjects in groups 1 and 2 were measured at 1h post-dosing except the second infusion on day 1 (12 h). Trough concentrations in serum were measured before the last infusion on the loading dose period (24 h) and then before each administration until day 10 (group 1) or day 9 (group 2). In addition, in group 2, mid-dose concentrations in serum were measured 24 hours after the administration of each dose on every day from day 4 to day 9. Thus a mid-dose concentration and a trough concentration (48 h after administration of the last dose) were measured for the subjects in group 2 after each 15 mg/kg infusion.

Analytical Procedure

The samples were collected in dry vials without anticoagulant, allowed to clot at room temperature for 20 min, and then centrifuged at

2500×g at 4°C. The resulting serum sample was transferred to another tube for storage at -80°C until assay.

Concentrations in serum were measured by high performance liquid chromatography according to a previously published method [14]. All compounds of the A2 group, A2-1 to A2-5, were measured in each sample allowing thus to calculate the proportion of each analog for each sample. All samples of the study have been already measured by a fluorescence polarization immunoassay (FPIA) and the results published elsewhere [13] in terms of a randomized comparison of serum teicoplanin concentrations following the daily and the alternate daily dosing [13]. For some specific sampling times, such as peaks and troughs, mean serum concentrations as measured by HPLC and FPIA were compared using the Student t-test for comparison of the means [15]. A p value<0.01 was considered as significant.

Results

Serum HPLC and FPIA concentrations

Peak and trough concentrations as measured by HPLC and FPIA [13] are shown in Table 1. All values as measured by FPIA are systematically superior to those measured by HPLC. Nevertheless for some of them, the difference is not significant whereas it is significant for others such as last peak concentrations in group 1 and group 2, and last trough concentration in group 2. The difference is not statistically significant for trough concentrations in group 1 (Table 1).

Ratio of different components of the A2 group between day 1 (D1) and D11, for peaks as well as for trough concentrations, it can be observed that the proportions of A2-1, A2-2 and A2-3 decrease, when those of A2-4 and A2-5 increase (Tables 2 and 3). The most pronounced differences are those related to A2-1 and A2-5. Thus, for residual values in group 1, the ratio of A2-2 decreases from 18%, when at the same time the ratio of A2-5 increases from 19%. These data are respectively -34% and +30% for trough concentrations in group 2. The variations are important too for analogs A2-2, A2-3 and A2-4, ranging from - 21% to+15%.

In what concerns the peak concentrations, for both groups, the variations are less pronounced, but go in a same way for each of the analog type.

Discussion

As already stated, an accumulation of teicoplanin occurs in

		HPLC	FPIA
	PEAKS		
group 1 (6 mg/kg)	1 st dose	32.8 ± 7.0	38.2 ± 6.7 (NS)
	3 rd dose	46.0 ± 8.8	58.2 ± 5.7 (NS)
	Last dose	43.5 ± 4.8	57.2 ± 5.2 (S) p< 0,002 [55-60]
Group 2 (12 then 15 mg/kg)	1 st dose	60.8 ± 15.3	71.4 ± 19.3 (NS)
	3 rd dose	106.2 ± 17.0	120.3 ± 18.4 (NS)
	last dose	92.5 ± 11.4	121.2 ± 15.4 (S) p < 0,001 [115-125]
	TROUGHS		
Group 1 (6 mg/kg)	Last dose	16.1 ± 1.25	16.0 ± 2.1 (NS)
Group 2 (12 then 15 mg/kg)	Last dose	14.1 ± 3.1	17.9 ± 3.5 (S) p < 0,002

(NS): not statistically significant difference with HPLC values; (S):statistically significant differences with HPLC values;
[x-y]: range of values

Table 1: Peak and trough concentrations in both groups as measured by HPLC and FPIA.

GROUP 1 (6 mg/kg, n=8) troughs		Mean% of total teicoplanin for each analog	S.D.	Delta D11 – D1
A2-1	D1	4.05	0.47	- 18
	D11	3.32	0.43	
A2-2	D1	45.8	1.6	- 11%
	D11	40.7	1.9	
A2-3	D1	7.7	1.1	- 21%
	D11	6.1	1	
A2-4	D1	17.1	1	+ 15%
	D11	19.6	1.6	
A2-5	D1	25.3	1.3	+19%
	D11	30.3	0.6	

Table 2: Teicoplanin trough concentrations at Day 1 (D1) and Day 11 (D11), for group 1 (upper) and group 2 (lower): percent of each analog of the A2 group, and delta between D11 and D1.

GROUP 1 (6 mg/kg, n=8) peaks		Mean % of total teicoplanin for each analog	S.D.	Delta D11-D1
A2-1	D1	5.7	0.7	- 12%
	D11	5.0	0.3	
A2-2	D1	50.5	1.0	- 5.3%
	D11	47.8	0.7	
A2-3	D1	8.8	0.5	- 10.2%
	D11	7.9	0.4	
A2-4	D1	16.0	0.8	+ 7.5%
	D11	17.2	0.8	
A2-5	D1	18.9	0.7	+ 17%
	D11	22.1	0.7	

Table 3: Teicoplanin peak concentrations at Day 1 (D1) and Day 11 (D11), for group 1 (upper) and group 2 (lower): percent of each analog of the A2 group, and delta between D11 and D1.

plasma after repeated administration [11]. The various components of teicoplanin differ between themselves by their lipophilicity, as indicated, for example, by their differing behavior in reversed phase HPLC [10]. This accumulation phenomenon, due to differences in lipophilicity, may be expected to vary from one analog to the other during the time course of a treatment. These different lipophilicities, together with the resulting different protein binding, could make one anticipate the existence of differences in their pharmacokinetic behavior and profile. Thus, when lipophilicity increases, Bernaregi et al. [12] reported a decrease in the volume of distribution and the total clearance of teicoplanin. They anticipated an increased accumulation for the more lipophilic compounds during the treatment but also between the 24h intervals separating two administrations.

We have evidenced this phenomenon in our work. There exist important differences in the evolution of the ratio to total A2-teicoplanin of the different analogs from the A2 group. The more lipophilic compounds A2-3 to A2-5 increase and the less lipophilic A2-1 and A2-2 decrease. This occurs with all trough and peak samples of the 24 patients all along the study (data not shown) to finally reach the D11 minus D1 differences shown in Tables 2 and 3, either for peaks or for residuals. It also occurs in the interval of two administrations of teicoplanin between the peak and the residual for both dosage regimens. This difference is more pronounced for residuals when compared with peaks because the 24 h or 48 h elapsed time between two administrations allowed a significant concentration in blood of the more lipophilic compounds. For the peaks, the injection of “fresh” teicoplanin should have led to more or less “re-initialized” ratios of the different analogs and thus minimize the delta seen for the residuals.

The extend of variations of the A2 group analogs that may occur

after teicoplanin administration are directly related to the initial ratio of the different analogs of the injected product. We have described here the variations resulting from the initial composition of the innovator (Targocid/Targosid). Up to now, there is no proof that the variations with time of the different analogs relative to the different marketed generics are equivalent, neither in blood nor in tissues. Colombo et al. [10] have reported that several teicoplanin generics products significantly differ in pharmaceutical quality from the originator on sub-group A2 distribution. The variations with time of the ratio of the various analogs of the A2 group seen in blood are likely to also exist in tissues. We have to keep in mind that the activities of the major components of teicoplanin against specific microbial species differ [11]. More, there is a synergistic activity of the different components of teicoplanin innovator in a certain ratio of these components [9]. Other ratios being different from those of innovator have not demonstrated to be synergic up to now.

Very recently, Rodriguez et al. [2] reported generic vancomycin to be less active than originator. More, it enriches resistant subpopulations of *S.aureus* after exposure in an animal model [16], as far as resistance is one of the natural consequences of suboptimal treatment of infection. This remains to be investigated with teicoplanin generics, as long as we do not know whether their analogs profiles are superimposable to those of innovator and have an equivalent tissue distribution and bactericidal activity.

Thus, as long as the effects of the differences of the A2 analogs have not been documented, concluding in bioequivalence of the different generics with the innovator is highly questionable.

Another matter of concern exits with the generics. The differences in concentrations obtained between FPIA and HPLC are well known [17,18] and, as occurred in our study, are systematically in favor of FPIA that gives higher concentrations than HPLC. FPIA is an immunoassay that uses sheep antibodies. It is likely to occur that the antibodies may have different affinity for the different analogs of teicoplanin and be more or less specific for one or the other analog, or many of them. Consequently, a variation with time of the different ratios of the analogs may lead to a non-linearity of the antigen-antibody responses of the immunoassay and explain, at last partially, the differences seen between HPLC and FPIA. The demonstration of this non-linearity may only be noticeable when there is a threshold level of accumulation or decrease of one or more analogs.

Most of the laboratories monitor teicoplanin levels by FPIA. According to the differences seen in the composition of the various generics [10], for one given ‘true’ concentration of total teicoplanin that would have been measured by HPLC, there is a risk of obtaining varying results as a function of the generics employed, since the distribution of components unpredictably changes with producer. Thus this represents an additional risk of having various marketed generics, the compositions of which are not similar between themselves and differs from the originator.

In conclusion, the complex pharmacokinetic profiles of the A2 group analogs of teicoplanin innovator remain to be demonstrated to be the same in the numerous generics that differ in composition. If this would not be the case, the incidences on the tissue penetration ratio, the bactericidal effects in blood and tissues, the resistance risks, and potential risky consequences on therapeutic monitoring by FPIA make that the presently assumed bioequivalence between generics and innovator remains highly questionable.

This underscore the need of redefining the regulatory rules before

the marketing authorization is given to certain antibiotics, as it has been recently suggested [19].

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