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PENICILLIN AT THE LATE STAGE OF LEPTOSPIROSIS: A RANDOMIZED CONTROLLED TRIAL

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SUMMARY

There is evidence that an early start of penicillin reduces the case-fatality rate of leptospirosis and that chemoprophylaxis is efficacious in persons exposed to the sources of leptospira. The existent data, however, are inconsistent regarding the benefit of introducing penicillin at a late stage of leptospirosis. The present study was developed to assess whether the introduction of penicillin after more than four days of symptoms reduces the in-hospital case-fatality rate of leptospirosis. A total of 253 patients aged 15 to 76 years with advanced leptospirosis, i.e., more than four days of symptoms, admitted to an infectious disease hospital located in Salvador, Brazil, were selected for the study. The patients were randomized to one of two treatment groups: with intravenous penicillin, 6 million units day (one million unit every four hours) for seven days (n = 125) and without (n = 128) penicillin. The main outcome was death during hospitalization. The case-fatality rate was approximately twice as high in the group treated with penicillin (12%; 15/125) than in the comparison group (6.3%; 8/128). This difference pointed in the opposite direction of the study hypothesis, but was not statistically significant (p = 0.112). Length of hospital stay was similar between the treatment groups. According to the results of the present randomized clinical trial initiation of penicillin in patients with severe forms of leptospirosis after at least four days of symptomatic leptospirosis is not beneficial. Therefore, more attention should be directed to prevention and earlier initiation of the treatment of leptospirosis.

KEYWORDS: Leptospirosis; Case-fatality rate; Prognosis; Death rate; Penicillin; Randomized clinical trial; Weil's Disease.

INTRODUCTION

Leptospirosis is a worldwide zoonotic disease caused by *Leptospira interrogans*[10,14,15,18]. The severe cases are characterized by a high frequency of jaundice, acute renal failure, bleeding complications, heart failure, cardiac arrhythmias and respiratory failure[6,10,17,19,20,22]. In Salvador, a large city located in the state of Bahia in Northeast Brazil, the disease is endemic with outbreaks occurring in close relation to the increase in the pluviometric precipitation[4,5,16]. The in-hospital case-fatality rate of leptospirosis in Salvador is approximately 12%[25]. This high death rate may be partly explained by the high frequency of severe disease among patients hospitalized with leptospirosis in Salvador[16,17]. The highest mortality risk has been described among patients who need mechanical ventilation or develop oliguric acute renal failure[19,23]. An increased mortality risk may also be due to a delay to start treatment[3].

BULMER reported in 1945 the first study about the effect of penicillin in patients with leptospirosis[3]. The benefit of the treatment was observed only when penicillin was introduced at an early stage of the disease. Other studies have failed to demonstrate benefit of penicillin in leptospirosis[8,15]. It is not clear, however, the influence of the stage of the disease on these negative results. Similar to BULMER[3], MCCLAIN *et al.*[21] showed evidence of a beneficial effect of the introduction of an antibiotic (doxycycline) at early stages of the leptospirosis. There is also evidence that doxycycline may prevent leptospirosis in highly exposed populations[26]. WATT *et al*., in a randomized, double-blinded clinical trial among patients with predominantly advanced leptospirosis, showed a smaller duration of hospital stay and fever in patients treated with penicillin[28]. In this study by WATT *et al*., however, it was not possible to assess the effect of penicillin on the case-fatality rate because there was no death case, both in the penicillin and the placebo groups. The present study was developed in Salvador specifically to assess whether the introduction of penicillin after at least four days of symptoms reduces the case-fatality rate of leptospirosis.

This work was carried out at the Clinical Epidemiology Unit of the Professor Edgard Santos Hospital, University of Bahia, Rua Augusto Vianna s/n, 6º Andar, Canela, 40110-060 Salvador, BA, Brasil, and Couto Maia Hospital from the Health Secretary of Bahia.

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METHODS

From August 1997 to July 1999, 253 patients admitted to Couto Maia Hospital, an infectious disease hospital located in Salvador-BA, were selected for the present study. Each patient signed a consent form, approved by the local institutional review board and was randomly assigned to be treated with (n = 125 patients) or without penicillin (n = 128) at a dose of 6 million units day (one million unit every four hours) for seven days. To be eligible for randomization the patient had to have more than four days of symptoms (i.e., late stage) and reach at least 26 points in the World Health Organization (WHO) probability score for leptospirosis[9]. The score was based on six clinical findings (headache, fever, conjunctival suffusion in both eyes, meningeal signs, myalgia and jaundice), azotemia and evidence of exposure to sources of leptospira (i.e., rats or contaminated water). In addition, the macroscopic slide test**,** the microagglutination test (MAT) and blood culture were performed for laboratory confirmation of leptospirosis. Exclusion criteria were: age below 15 years, history of allergy to penicillin, immunodeficiency, previous history of nephropathy or cardiomyopathy, diabetes mellitus and pregnancy.

The patients were submitted to a list of laboratory exams at hospital admission that were repeated at least in the days 3 ± 1 and 7 ± 1 after admission. The list consisted of hemogram, bilirubins, aminotransferases (AST, ALT), prothrombin time, urea, creatinine, sodium, potassium, albumin, urinalysis and serologic tests for leptospirosis. The blood sample for bacteriological identification of leptospira was collected at admission. The MAT and blood culture for leptospira were performed at Centro de Pesquisas Gonçalo Moniz da Fundação Oswaldo Cruz/FIOCRUZ. The macroscopic slide test was performed at the Laboratório Central de Saúde Pública Gonçalo Moniz/LACEN. The MAT was considered positive when there was at least four-fold increase in the reciprocal titre between paired serum samples or when the reciprocal titre was greater than 800 in one or more serum samples. The MAT was defined as probable when the reciprocal titre was between 100 and 800. For 127 patients chest radiographs were performed independently of clinical manifestations. The radiographs were independently analyzed by two pneumologists who were blinded to any other data of the patients. The inter-observer agreement between the pneumologists was high (kappa = 0.7). Radiographs with discordant diagnoses were submitted to a radiologist for independent opinion. The discordance was resolved by consensus among the three observers.

Statistical Analysis. Intention to treat analysis was used. The t test and chi-square test for independent groups were used to compare differences in quantitative and qualitative variables, respectively. Logistic regression was used to adjust the association between treatment (groups with and without penicillin) and death rate for base-line difference in the leukocyte count. To obtain adjusted odds ratio, two dummy variables were defined for leukocyte count: 10,001 to 20,000 per mm^3 and 20,001-plus. Leukocyte count ≤ 10,000 was used as the referent.

RESULTS

The macroscopic slide test was performed for 252 of 253 patients, being positive in 247. The MAT was performed for 65 patients being positive for 45 and probable for 8. Positive hemocultures were found in 17 out of 21 patients. In one patient the diagnosis was based only in clinical and epidemiological findings.

Table 1 shows the baseline characteristics of the patients. Icterus at hospital admission was present in 94.1% (238/253). The penicillin and

Table 1
Baseline characteristics of the patients

Characteristic	Penicillin N = 125	Control N = 128	p value
Male, n/N (%)	110/125(88.0)	117/128(91.4)	0.372
Icteric, n/N (%)	118/125(94.4)	120/128(93.8)	0.827
Altered chest radiograph at admission, n/N (%)	16/61(26.2)	17/66(25.8)	0.952
Shortness of breath	4/125(2.2)	8/128(6.3)	0.254
Creatinine ≥ 1.5 mg/dl, n/N (%)	112/122(91.8)	116/127(91.3)	0.895
Creatinine > 3.0 mg/dl, n/N (%)	40/122(32.8)	35/127(27.6)	0.369
Bleeding*	39/117(33.3)	39/124(31.5)	0.755
Age in years, mean ± SD (N)	35.8 ± 13.9(125)	35.1 ± 13.1(128)	0.700
Duration of symptoms in days, mean ± SD (N)	6.6 ± 1.8(125)	6.5 ± 1.6(128)	0.349
Body temperature in Celsius scale, mean ± SD (N)	37.0 ± 0.9(118)	37.0 ± 0.7(120)	0.546
Hematocrit, mean ± SD (N)	34.0 ± 5.5(116)	34.5 ± 5.0(124)	0.483
Leucocyte count, mean ± SD (N)	16325 ± 6436(120)	14584 ± 4594(125)	0.016
Total bilirubin in mg/dl, mean ± SD (N)	20.7 ± 11.9(113)	22.2 ± 14.4(113)	0.370
Creatinine in mg/dl, mean ± SD (N)	4.6 ± 2.3(123)	4.3 ± 2.0(127)	0.398
Sodium in mEq/l, mean ± SD (N)	134.0 ± 6.5(100)	132.2 ± 5.8(92)	0.354
Potassium in mEq/l, mean ± SD (N)	3.4 ± 0.7(102)	3.4 ± 0.7(98)	0.673
Albumin in g/dl, mean ± SD (N)	3.0 ± 0.5(95)	3.0 ± 0.6(107)	0.764

* the source of bleeding was identified as respiratory or gastrointestinal in 96.2% of the cases

the control groups were similar regarding the presence of jaundice, gender, age, duration of the disease and body temperature. Laboratory findings at admission were similar between the groups, except for the leukocyte count (higher in the penicillin group). All laboratory exams performed around the 3rd and the 7th hospitalization days were similar between the penicillin and the control groups (the data are not shown in the table). Renal failure at admission, defined as serum creatinine ≥ 1.5 mg/dL, was observed for 91.6% of the patients. The percent of patients with renal failure was similar between the penicillin (91.8%) and the control (91.3%) groups. Creatinine > 3.0 mg/dL at admission was present in approximately 32.8% and 27.6% of the patients randomized to the penicillin and control groups, respectively (p = 0.369). Significant differences were also not observed between the penicillin and the control groups regarding the percentages of patients with shortness of breath and bleeding at hospital admission. Among 127 patients who had a chest radiograph performed, independently of the clinical manifestations, radiological alterations were detected in 33 (26%). The percentage of radiographs with alterations was similar between patients of the penicillin (26.3%) and the control (25.8%) groups. Alveolar infiltrate was the predominant radiologic pattern being observed in 12 of the 16 radiographs with alterations in the penicillin group and in 12 of the 17 in the control group.

There were 23 cases of death. These deaths were attributed to acute renal failure, respiratory failure and multiple organ failure in 14 (60.9%), 1 (4.3%) and 8 (34.8%) patients, respectively. Approximately 44% of the death cases occurred within the first three days of hospitalization. These early deaths corresponded to 46.7% (7/15) and 35.7% (3/8) of the fatal cases in the groups penicillin and control, respectively. The in-hospital case-fatality rate (Table 2) was higher in the penicillin group (12.0%; 15/125) than in the control group (6.3%; 8/128); the difference, however, was not statistically significant (p = 0.112). The odds of death remained higher in the penicillin than in the control group, even after taking into account baseline difference in the leukocyte count (odds ratio = 1.94, 95% confidence interval = 0.78-4.80). All deaths were observed among icteric patients. The length of hospital stay was similar between the penicillin (8.9 ± 3.9 days) and the control (8.8 ± 3.6 days) groups (the data are not shown in the table). In an analysis restricted to patients who remained alive during hospitalization, there was also no important difference between the groups in the length of hospital stay; 9.4 ± 3.5 and 9.0 ± 3.4 days for the penicillin and the control groups, respectively. Peritoneal dialysis was the modality of renal replacement therapy for acute renal failure. It was used for 28% (35/125) of the patients in the penicillin group and 18% (23/128) in the control group (p = 0.058).

Table 2
Unadjusted and logistic-regression adjusted associations between demographic characteristics and in-hospital case-fatality rate

Treatment group	Death			Odds ratios (95% CI)	
	Yes	No	% death	Unadjusted	Adjusted*
Penicillin	15	110	12.0	2.04 (0.83-5.01)$^{\phi}$	1.94 (0.78-4.80)$^{\uparrow}$
Control	8	120	6.3	referent	referent

*odds ratio adjusted for leukocyte count at admission; $^{\phi}$p = 0.118; $^{\uparrow}$p = 0.154

DISCUSSION

The results of the present work do not support the idea that penicillin has a beneficial effect in patients with leptospirosis when it is started after the fourth day of the disease. Although the difference was not statistically significant, the case-fatality rate was greater in the group randomized to penicillin. In addition, the hospital stay was similar between the groups. It is also important to note that according to the reports from the Health Secretary of Bahia relative to the period 1988-1999, the in-hospital case-fatality rate of leptospirosis in Salvador is around 12 percent[25]. This case-fatality rate is similar to that found in the group treated with penicillin in the present study and superior to the one observed in the control group.

To our knowledge the only randomized clinical trial that reported benefit with penicillin when the treatment was started late in the course of the disease was the study by WATT *et al*.[28]. In order to compare our results with those reported by WATT *et al.*, however, it is important to take into account the severity of the disease and the outcome used to assess treatment efficacy. In the study by WATT *et al*., only half of the sample had elevated levels of bilirubin and icterus by physical examination was reported for approximately 45%. By contrast, in the present study more than 90 percent of the patients were icteric. WATT *et al*. observed a shorter duration of fever and hospital stay among the patients on penicillin than among those from the control group. None of the patients died, what is consistent with the fact that the group was comprised of patients with less severe forms of leptospirosis as compared to the present study. Since there was no fatal case, it is not possible to conclude by this previous study whether the introduction of penicillin at late stage of leptospirosis is related to reduction in the case-fatality rate. In the trial by WATT *et al*., and in other previous studies, the use of antibiotics was also associated with a faster disappearance of the leptospiruria[2,8,21,28]. There is no evidence, however, that this finding is associated with a better prognosis of the disease.

Even though in the present study the treating physicians were not blind to the patient assignment, the fact that the main outcome (death) was a very objective one reduces the possibility of bias. Our data suggesting that penicillin do not reduce the probability of death when it is introduced late in the course of leptospirosis provide additional support to the importance of an earlier start of the antimicrobial treatment in suspect cases. In addition, the use of penicillin did not decrease the need for dialysis treatment. In fact there was a tendency for a higher frequency of patients who needed dialysis in the penicillin group. DAHER & NOGUEIRA studied a sample of 43 patients from the state of Ceará with acute renal failure due to leptospirose and a high mean duration of symptoms and have also not found a beneficial effect of penicillin in preventing adverse outcomes[7]. Experimental investigations in animals and human studies have shown evidence that the use of antimicrobial agents soon after the appearance of symptoms improves the prognosis of leptospirosis[2,21]. In addition, there is evidence favoring the use of antibiotics in persons exposed to the sources of leptospira[13,24,26]. These findings are consistent with the idea that once established the tissue lesions by the leptospira the antibiotic cannot change the course of the disease.

The present sample was comprised of patients with severe forms and late stage of leptospirosis. Therefore, our results cannot be generalized

to the whole population of patients with this disease. It is important to note that approximately 44% of the deaths occurred within the first three days of hospitalization with a slight preponderance of early death among patients randomized to penicillin. It is plausible to hypothesize that because of the severity of the disease some patients randomized to penicillin died before receiving the benefit of the treatment. There is also the possibility that the clinical manifestation of leptospirosis worse after the initiation of antibiotic therapy due to the development of Jarisch-Herxheimer reaction[27]. However, in the present study we have not observed classical manifestations of Jarisch-Herxheimer in none of the patients treated by penicillin.

In conclusion, the results of the present randomized clinical trial in patients with severe forms of leptospirosis do not support the hypothesis that the initiation of penicillin after at least four days of symptomatic disease is beneficial. Our data call attention to the need for a greater emphasis on the prevention of leptospirosis and identification of patients with symptomatic disease at an earlier stage when the initiation of antibiotic therapy seems to be efficacious in decreasing the risk of death.

RESUMO

Penicilina na fase avançada da leptospirose: um ensaio clínico randomizado

Existe evidência de que o início precoce do tratamento com penicilina reduz a letalidade da leptospirose e de que a quimioprofilaxia é eficaz em pessoas expostas às fontes de infecção. Os dados existentes, contudo, são inconsistentes quanto ao benefício de iniciar penicilina na fase tardia da leptospirose. O presente estudo foi desenvolvido para avaliar se a introdução de penicilina após mais de quatro dias de sintomas reduz a letalidade da leptospirose. Um total de 253 pacientes entre 15 e 76 anos de idade, com leptospirose avançada, i.e., mais de quatro dias de sintomas, admitidos em um hospital de doenças infecciosas localizado em Salvador, Brasil, foram selecionados para o estudo. Os pacientes foram randomizados para um dos seguintes grupos de tratamento: com penicilina intravenosa, 6 milhões de unidades/dia (um milhão de unidades cada quatro horas) por 7 dias (n = 125) e sem penicilina (n = 128). O evento principal foi morte durante o período de internamento. A letalidade foi aproximadamente duas vezes maior no grupo tratado com penicilina (12%; 15/125) do que no grupo de comparação (6,3%; 8/128). Esta diferença seguiu direção oposta a da hipótese do estudo, porém não alcançou significância estatística (p = 0,112). A duração do internamento foi similar entre os grupos de tratamento. De acordo com os resultados do presente ensaio clínico randomizado o uso de penicilina não é benéfico em paciente com leptospirose quando iniciado com pelo menos quatro dias após o início dos sintomas. Portanto, maior atenção deve ser dada à prevenção e ao inicio mais precoce do tratamento da leptospirose.

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