Toxicological Assessment of Beta-lapachone on Organs from Pregnant and Non-pregnant Rats

Edvaldo Rodrigues de Almeida1,*, Flávia Raquel Santos Lucena1, Camilla Vila Nova Soares Silva1, Wilson da Silva Costa-Junior1, Jouse Bezerra Cavalcanti2, Gerald Bosco Lindoso Couto2, Luiz Lúcio Soares da Silva1, Diógenes Luís da Mota3, Alex Benício da Silveira4, Samuel Daniel de Sousa Filho5 and Aldo Cezar Passilongo da Silva6

1Laboratory of Evaluation of Psychobiobioactive Drugs and its Toxicology, Federal University of Pernambuco, 50670-901 Recife PE, Brazil
2Odontology Department, Federal University of Pernambuco, 50670-901 – Recife PE, Brazil
3Department of Histology and Embryology, Federal University of Pernambuco CEP 50670-901 Recife PE, Brazil
4Laboratory of Hematology and Biochemist of Pharmacy Department, Federal University of Pernambuco 50670-901, Recife – PE, Brazil

Keywords: β-lapachone; biochemical and hematological study; histological study; Wistar rats.

INTRODUCTION

Naphthoquinones are widely distributed in the plant kingdom. Their molecular structures endow them with redox properties, which can intervene in biological oxidative processes (Menna-Barreto et al., 2005; Silva et al., 2003; Portela et al., 1996). B-Lapachone (beta-lap) is a lipophilic o-naphthoquinone isolated from the bark of the lapacho tree, through lapachol, that is native to Central and South America and has antibiotic/antineoplastic potential (Santana et al., 1968; Silva et al., 2003). Initial observations proved its capability for inhibiting the growth of Yoshida sarcoma and Walker 256 rat carcinosarcoma (Santana et al., 1968; Dubin et al., 2001; Pu et al., 2004), and against various cancer cell lines such as human ovarian and prostate tumors (Li et al., 1999; Ravelo et al., 2004; Duvoix et al., 2004; Kumi-Diaka et al., 2004; Lee and Lee, 2004). Its mechanism of action includes the inhibition or activation of topoisomerase I and II in a manner that is distinct from those of other topoisomerase inhibitors (Chen et al., 2004; Park et al., 2005). In recent years interest in these substances has intensified, not only due to their importance in vital biochemical processes, but also due to their more and more frequent presence in varied pharmacological studies, mainly in the levels of the cellular respiratory chain. Beta-lap inhibited DNA synthesis in Trypanosoma cruzi as well as topoisomerases I and II in different cells (Menna-Barreto et al., 2005; Woo and Choi, 2005; Perez-Sacau et al., 2005). These enzymes are essential for maintaining DNA structure. Advances in knowledge on apoptosis (‘programmed cell death’) and necrosis provided useful information for understanding the mechanism of cytotoxicity of beta-lap (Tagliarino et al., 2001; de Witte et al., 2004). The cytotoxicity of this naphthoquinone is related to inhibition of topoisomerases and the induction of apoptosis (Dubin et al., 2001; Oliviera-Brett et al., 2002). It has been shown that beta-lap is active against T. cruzi and its mode of action is associated with the generation of free radicals (Portela et al., 1996; Abreu et al., 2002; de Witte et al., 2004; Zielinska-Park et al., 2004; Villamil et al., 2004). The objective of this paper was to demonstrate a possible toxic effect of beta-lap on Wistar rats, through its action on pregnant rats, and in treatment for 21 days, for histological, hematological and biochemical analyses. Since there may be a risk to pregnant patients with this anticancer drug.

MATERIAL AND METHODS

Animals. Adult Wistar rats of both sexes (160–240 g), 2 months of age at the beginning of the experiment, were used. The animals were obtained from the vivarium of the Antibiotic Department of the Federal University of Pernambuco and were housed in groups of ten per
E. RODRIGUES DE ALMEIDA ET AL.

displacement on day 19 of gestation. A laparatomy was performed and the uterus and ovaries were removed. Resorptions (embryotoxicity/fetotoxicity) were counted and viable implants were examined. The number of live/dead fetuses, viability, growth and deformity of newborn and maternal weight gain were recorded (Almeida et al., 2000; Barrow, 2003; Jelinek, 2005).

Long-term administration effects (21 days). Male and female Wistar rats were studied. All were submitted to administration of 40, 80 and 160 mg/kg (i.p.), ten animals for each dose, for 21 days. The blood levels of total bilirubin, total cholesterol, creatinine, urea, glucose, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), gamma glutamyl transferase (gamma GT) and alkaline phosphatase (AP) were measured at 0 and 21 days. A colorimetric method was used for each one with the Doles® diagnostic system. At the end, each animal was killed by cervical displacement 24 h after the last treatment and then the liver, kidneys and spleen were removed for histological study (Casarett and Doull, 1975; Oga, 2003).

Statistical evaluation. The data were submitted to variance analysis (ANOVA). Posthoc comparisons between individual treatments and controls were made using Student’s t-test. The results were considered significant when \( p < 0.05 \) and \( p < 0.01 \) were obtained (Siegel, 1975).

RESULTS

Teratogenic and abortive activity in pregnant rats

As observed in Tables 1 and 2, the beta-lap at the three doses (40, 80, and 160 mg/kg) administered on days 7–12

Table 1. Effect of beta-lap in the period of days 1–6 and days 7–12 of gestation

<table>
<thead>
<tr>
<th>Analysed parameter</th>
<th>Control group (saline + Tween 80)</th>
<th>Dose (40 mg/kg)</th>
<th>Dose (80 mg/kg)</th>
<th>Dose (160 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation of the maternal average weight</td>
<td>73 ± 2.42</td>
<td>44.8 ± 8.7*</td>
<td>43 ± 5.52*</td>
<td>39 ± 5.03*</td>
</tr>
<tr>
<td>Fetal weight</td>
<td>1.59 ± 0.01</td>
<td>1.18 ± 0.12*</td>
<td>1.13 ± 0.03*</td>
<td>1.08 ± 0.14*</td>
</tr>
<tr>
<td>Weight of the placenta</td>
<td>0.41 ± 0.01</td>
<td>0.34 ± 0.02*</td>
<td>0.32 ± 0.04*</td>
<td>0.26 ± 0.01*</td>
</tr>
<tr>
<td>Resorption index (RI)</td>
<td>0.0</td>
<td>7.27*</td>
<td>16.66*</td>
<td>18.36*</td>
</tr>
<tr>
<td>Malformations</td>
<td>0.0</td>
<td>4.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Total number of corpora lutea</td>
<td>9 ± 0.81</td>
<td>10 ± 0.51</td>
<td>10.3 ± 0.71</td>
<td>10 ± 0.82</td>
</tr>
<tr>
<td>Number of viable embryos</td>
<td>60</td>
<td>5</td>
<td>48</td>
<td>49</td>
</tr>
</tbody>
</table>

Values are mean ± SD for 10 animals; * \( p < 0.05 \); ** \( p < 0.01 \) vs control group. Student’s t-test followed by one-way variance analysis (ANOVA). Variation of the average maternal weight is the difference between the fetus controls for fetuses of the treated groups.

Table 2. Type of malformations observed in the embryos on days 1–6 and days 7–12 of gestation

<table>
<thead>
<tr>
<th>Fetal observation and type of anomaly</th>
<th>Dose (40 mg/kg)</th>
<th>Dose (80 mg/kg)</th>
<th>Dose (160 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterocelia</td>
<td>x</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>Absence of tail</td>
<td>x</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>Absence of members</td>
<td>x</td>
<td>–</td>
<td>X</td>
</tr>
<tr>
<td>Bifid spine</td>
<td>–</td>
<td>x</td>
<td>X</td>
</tr>
<tr>
<td>Sindacty</td>
<td>x</td>
<td>x</td>
<td>X</td>
</tr>
</tbody>
</table>

X, presence of malformation; –, absence of malformation.
of gestation led to fetal toxicity. Furthermore, malformations of the implant and viable fetus were observed in the animals killed on day 19 of pregnancy. The malformations presented were enterocelia, absence of tail, absence of members, bifid spine and sindactly, beyond resorption in the two periods of application in all the doses used (Table 2). A weight decrease of the malformed fetus in relation to the viable fetus also occurred in a significant form (Table 1). The dose of 160 mg/kg produced the most malformation and abortive activity.

**Histological studies**

The beta-lap at a dose of 160 mg/kg, promoted alteration in the spleen structure of the experimental group and showed enlarged follicles in the white pulp when compared with the control group. A relatively high frequency of hypertrophy in the follicles was observed in the mantle zone as well as in the perifollicular (marginal) zone (Fig. 1). No changes in the liver structure were observed in the experimental animals. Under light microscopy, the parenchyma (hepatocytes) and stroma components were well preserved. The beta-lap, at the same dose, did not produce histological alterations in the kidneys of the animals of the experimental group.

**Hematological and biochemical studies**

Beta-lap at doses of 40, 80 and 160 mg/kg promoted a significant increase in the number of total leukocytes, as well as in the monocytes and segmented blood cells in the animals of the experimental group (Table 3). At the biochemical level, the beta-lap promoted an increase in glutamate pyruvate transaminase (GPT), gamma glutamyl transferase (gamma GT) and alkaline phosphatase (AP) in relation to animals of the control group (Table 4).

![Figure 1. Microphotography of histological preparation of the spleen in the control group (B) increased 10× and experimental group (A), increased 10×. The histological analyses of the spleen structure of the experimental group (A), showed enlarged follicles in the white pulp when compared with control group (B). In these follicles, a relative hypertrophy was observed in the mantle zone as well as in the perifollicular (marginal) zone. Enlarged follicles were observed in the white pulp of the spleen of rats of the experimental group. In the follicles, hypertrophy was seen at high frequency in the mantle zone at the doses used.]
DISCUSSION

Beta-lap, with its molecular structure, presents redox properties that intervene in biological oxidative processes (Menna-Barreto et al., 2005). It also presents inhibition of development of Yoshida sarcoma and Walker 256 rat carcinosarcoma (Santana et al., 1968; Pu et al., 2004). In humans, it presents an action against ovarian and prostate tumors (Ravelo et al., 2004; Duvoix et al., 2004; Kumi-Diaka et al., 2004; Lee and Lee, 2004). Its mechanism of action includes the inhibition or activation of topoisomerase I and II in a more distinct manner than those of other topoisomerase inhibitors (Chen et al., 2004; Park et al., 2005). In recent years interest in these substances has intensified, not only due to their importance in vital biochemical processes, but also to their more and more frequent presence in varied pharmacological studies, mainly in the levels of the cellular respiratory chain. Beta-lap inhibited DNA synthesis in Trypanosoma cruzi as well as topoisomerases I and II in different cells (Menna-Barreto et al., 2005; Woo and Choi, 2005; Perez-Sacau et al., 2005). These enzymes are essential for maintaining DNA structure. Advances in knowledge on apoptosis (‘programmed cell death’) and necrosis provided useful information for understanding the mechanism of cytotoxicity of beta-lap (Tagliarino et al., 2001; de Witte et al., 2004). However, there is little knowledge regarding its toxicity in long-term treatment. The study of pregnant rats treated intraperitoneally (i.p.) with doses of 40, 80 and 160 mg/kg demonstrated anatomical alterations in the fetus. The beta-lap showed abortive and teratogenic action in pregnant rats (Tables 1, 2). This observation indicates that beta-lap acts at the beginning of egg division and during its implantation. The physiopathological mechanism of this effect is probably the same as the one that promotes its cytotoxic action, through the enzymes that act in the DNA chain, as well as for its action in the induction of apoptosis. On the other hand, the significant increase in the total leukocytes, segmented and monocytes could demonstrate a stimulant action of the beta-lap in the immunological system (Table 3). Histological analysis of the spleen structure showed enlarged follicles in the white pulp (Fig. 1). These data can explain the response of an increase of total leukocytes, segmented and monocytes in the blood circulation during the treatment. The data found in spleen and the immune-competent cells of the blood can be another action of beta-lap against cancer cells. However, further studies on this effect will have to be carried out in the future. The significant increase of gamma GT, GPT and AP levels can explain damage in the liver, mainly in the biliary duct, when associated with an increase in total bilirubin in blood circulation, an effect that was not observed in the histology (Table 4). In the histology and in the biochemistry of kidney (creatinine and urea) no alteration was observed in the treatment with beta-lap at the doses used. The data for the histology of spleen and the increase in the white cells of the blood could possibly indicate a new action of beta-lap as an immunostimulant and will be the subject of future studies in our laboratory.

Acknowledgements

The authors wish to express their thanks to Conselho Nacional de Pesquisa (CNPq) and Universidade Federal de Pernambuco (UFPE) for the financial assistance.

REFERENCES


Copyright © 2009 John Wiley & Sons, Ltd.


