

## EFFECT OF RIBAVIRIN ON EPIDIDYMAL SPERM COUNT IN RAT

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**Abstract :** Ribavirin (1- $\beta$ -D-ribofuranosyl-1, 2, 4-triazole-3-Carboxamide) is a potent inhibitor of inosine monophosphate dehydrogenase, used widely as an antiviral drug. Although it has been reported as a teratogen, its effect on spermatogenesis is not known. Male Wistar rats were segregated into 24 groups of 5 in each. Six groups were treated with water, 6 groups with 20 mg/kg, another 6 groups with 100 mg/kg and remaining 6 groups with 200 mg/kg for 5 days at intervals of 24 h (i.p.). Animals were anaesthetized at 14, 28, 35, 42, 70 and 105 days following the last exposure, laparotomy was conducted, epididymis was removed, minced in 1 ml phosphate buffered saline (PBS, pH 7.2), filtered and stained with 1% aqueous eosin Y. An aliquot was taken in haemocytometer, diluted in PBS and charged into Neubauer's chamber. Spermatozoa were counted in 8 squares except the central, and multiplied by  $5 \times 10^4$ . Data were analysed by Mann-Whitney "U" test. Ribavirin significantly decreased the sperm count in a dose and time dependent pattern and showed a recovery by day 105 except at 200 mg/kg. Ribavirin is reversibly cytotoxic to germ cells and decreases the production of spermatozoa.

**Key words :** *ribavirin* sperm count epididymis rat

### INTRODUCTION

Ribavirin (1- $\beta$ -D-ribofuranosyl-1, 2, 4-triazole-3-Carboxamide) is a synthetic purine nucleoside analogue widely used as an antiviral drug (1). It is a potent inhibitor of inosine monophosphate dehydrogenase (IMPd) activity which catalyses the conversion of inosinate to xanthylate (2). It inhibits a variety of DNA and RNA viruses such as influenza, herpes, measles, chicken

pox, respiratory syncytial virus (RSV) and hepatitis (3, 4, 5). Further, it is a most suitable drug against RSV (6) and hepatitis (7) and its combination with interferon alfa is promising for the treatment of the latter (8).

In view of its clinical efficacy, several studies were performed to evaluate the toxicity profiles. Consequently, it was reported that ribavirin produces craniofacial

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and limb deformities in rats and hamsters (9, 10). In addition to these developmental anomalies, it also depressed the DNA synthesis and induced necrotic cell death in mice (11). It is cytotoxic in bone marrow of monkeys producing dose dependent anaemia and thrombocytosis associated with marrow erythroid hypoplasia and megakaryocyte hyperplasia (12). In patients, aerosol therapy produces dyspnoea, chest soreness, worsening of respiratory status, bacterial pneumonia and deterioration of pulmonary functions (13). However, its toxicity on germ cells is not clear although Hoffmann et al (14) reported that ribavirin has no effect on fertility in male rats. This study was therefore undertaken to evaluate the cytotoxic effect of this drug on germ cells using the parameter of epididymal sperm count in the rat.

## METHODS

Male rats of Wistar strain (11–13 wk) were procured from animal house of Kasturba Medical College, Mangalore. They were housed in polypropylene cages with paddy husk bedding under standard laboratory conditions. All animals had access to laboratory chow and water *ad libitum*.

Powdered form of ribavirin (Virazole, ICN Pharmaceuticals, Inc., California, Lot no 94 J02) was employed in this study. Three dose levels, 20, 100 and 200 mg/kg were selected and the drug was dissolved in water for injection just before use. Animals were segregated into 24 groups of 5 animals in each. Six groups were treated with water (0.1 ml/animal), 6 groups with 20, another 6 groups with 100 and remaining 6 groups

were treated (i.p.) with 200 mg/kg ribavirin for 5 days at intervals of 24 h. Since ribavirin is known to be well absorbed from the peritoneal cavity as advocated by previous authors (15), we have selected this route of administration. Animals were anaesthetized (Pentobarbital sodium, 45 mg/kg) at 14, 28, 35, 42, 70 and 105 days following the last exposure. Laparotomy was conducted and the reproductive organs were exposed. The epididymis from one side was removed and minced in 1 ml phosphate buffered saline (PBS, pH 7.2) and the suspension was filtered through 80  $\mu$  nylon mesh. One drop of 1% aqueous eosin Y was added to the filtrate and kept for 30 minutes (16). An aliquot of the suspension was taken in white blood cell pipette (haemocytometer) up to 0.5 mark and then diluted in PBS up to mark 11. The dilution was mixed thoroughly and charged into Neubauer's chamber. The sperm count was conducted according to the standard procedure (17) in 8 squares of 0.1 cm<sup>2</sup> each except the central erythrocyte area. The total count was then multiplied by correction factor,  $5 \times 10^4$ .

For each group, mean and SE were calculated and the data were analysed by Mann-Whitney 'U' test using SPSS software package, version 7.

## RESULTS

Following the exposure of ribavirin, the sperm concentration was declined in epididymal suspension (Table I). Sperm count was decreased at two higher dose levels at day 14 itself. However, the test agent at all three doses depressed ( $P < 0.01$ ) the spermatogenesis on days 28, 35 and 42 compared with the respective controls. On

TABLE I: Epididymal sperm count ( $\times 10^6$ ) in control and ribavirin treated rats.

Drug/dose	Sample time (d)					
	14	28	35	42	70	105
Control	54.72 $\pm$ 2.50	57.34 $\pm$ 2.15	55.19 $\pm$ 1.82	57.41 $\pm$ 2.17	56.82 $\pm$ 2.47	60.15 $\pm$ 2.12
Ribavirin 20 mg/kg	50.63 $\pm$ 1.02	46.54 $\pm$ 1.55**	42.46 $\pm$ 1.81**	48.47 $\pm$ 1.37**	55.35 $\pm$ 1.70	61.32 $\pm$ 1.95
Ribavirin 100 mg/kg	43.60 $\pm$ 0.96** <sup>aaa</sup>	36.73 $\pm$ 1.37** <sup>aaa</sup>	31.91 $\pm$ 1.92** <sup>aaa</sup>	44.56 $\pm$ 0.52** <sup>aa</sup>	56.21 $\pm$ 0.88	63.88 $\pm$ 2.37
Ribavirin 200 mg/kg	40.72 $\pm$ 1.30** <sup>bb</sup>	31.28 $\pm$ 0.72** <sup>bbcc</sup>	24.41 $\pm$ 1.62** <sup>bbcc</sup>	40.86 $\pm$ 1.34** <sup>bbcc</sup>	41.19 $\pm$ 1.60** <sup>bbcc</sup>	55.17 $\pm$ 1.35 <sup>bc</sup>

Each treatment at particular time point represents mean  $\pm$  SE for 5 animals. \*\*P<0.01 control versus treated; <sup>a</sup>P<0.05, <sup>aa</sup>P<0.01, ribavirin 20 versus 100 mg/kg; <sup>b</sup>P<0.05, <sup>bb</sup>P<0.01, ribavirin 20 versus 200 mg/kg; <sup>c</sup>P<0.05, <sup>cc</sup>P<0.01, ribavirin 100 versus 200 mg/kg.

day 70, two lower doses had no significant effect on the sperm production, whereas the higher dose continued to affect it (Table I). Despite this, the recovery to control limit was observed by day 105.

Dose-response followed a negative linear relation except at days 70 and 105. Hence, the sperm production decreased in a dose dependent pattern. Time-response relationship followed parabolic pattern indicating the maximum toxicity at day 35.

## DISCUSSION

Drugs depress the spermatogenesis in mammals due to cytotoxicity (18) which results in death of immature germ cells in the seminiferous epithelium (19). Due to cell removal, total cells available for spermatogenesis also reduce and hence depressing the daily sperm production. Present results thus indicate that ribavirin inhibited spermatogenesis probably affecting the cell multiplication and differentiation. Ribavirin is known to inhibit cell proliferation and synthesis of nucleic acids in the limb buds of mouse embryo (11).

Depression of nucleic acid synthesis is known to result in apoptosis and hence eliminating the germ cells in mitomycin C treated mouse (20). Ribavirin or its metabolites bind to IMPd thereby inhibiting guanylate synthesis (21), a process that hinders the cell growth and induces the cell death (22). Hence, it is possible that destruction of germ cells takes place in the testis which consequently decreases the sperm production.

Following the treatment of ribavirin, the sperm count was reduced in a negative linear relation between days 14 to 35 indicating a gradual raise of toxicity through time. The extreme toxicity at day 35 indicates that the sperm samples obtained at this sample time were spermatogonia during exposure and this class of germ cells is more sensitive to this drug. However, the decreased sperm count at other sample times indicates that other cell types were also affected. Previously, it was reported that even human therapeutic dose level (20 mg/kg) when given by multiple treatments depressed the erythrocyte count in mice (15). In the present study, ribavirin

at the same dose level was found to affect the spermatogenesis. In erythrocytes, binding of ribavirin is well recognised and likely factor for the extended  $\frac{1}{2}$  ( $\beta_2$ ) beyond 40 days. This would have been a likely reason for the sperm suppression up to day 42 and returned to normal levels at day 70 with 2 lower doses. However, single dosage of even slightly higher dose (25 mg/kg) did not show any teratogenic potential in mice (11). This indicates that this drug or its metabolites produce combined effect on germ cells when administered by multiple treatments as seen in the case of hydroxyurea (23). A dominant lethal test conducted in rats (14) revealed that ribavirin does not affect the  $F_1$  generation, even though the foetal weight was decreased. Authors have concluded that, the effects were biological chance events. They have neither studied the sperm morphology nor

the sperm count. We have conducted a study on sperm count which decreased to around 50% of control limit on day 35 at 200 mg/kg. Since, decreased sperm count has positive correlation with infertility (24) ribavirin may affect the fertility parameters.

The present study also confirms the previous hypothesis that ribavirin is a selective cytotoxin to rapidly dividing cells (11). These authors have found that it induces necrosis of limb bud cells in mouse embryo. Similarly, ribavirin acted as a cytotoxin to bone marrow, where this drug affected the maturation of erythrocytes and caused intracellular changes (12, 15). We conclude that ribavirin or its metabolites act as cytotoxins in rat testis and affect the epididymal sperm count in a dose and time dependent manner.

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