Hypolipidemic, hypoprostatic and pharmacological activity of oxohexaene polyene antibiotic (HA-1-92)

*S R Naik & J Harindran
Research & Development division, Hindustan Antibiotics Limited, Pimpri, Pune 411 018, India.
Received 28 July 1999; revised 31 March 2000

HA-1-92, a new oxohexaene polyene macrolide showed significant hypolipidemic and hypoprostatic activity both in rat and rabbit models. HA-1-92 did not elicit significant effect on blood pressure or respiration in anaesthetized rat or on various isolated tissue preparation studies.

During soil screening programme in search of new antifungal antibiotics, a Streptomyces culture, named CDRIL-312, obtained from Central Drug Research Institute (CDRIL), Lucknow, India produces an oxohexaene polyene antibiotic, which has been designated as HA-1-92. Its fermentation, purification and antimicrobial activity has been reported earlier. Lyposomal preparation of HA-1-92 and its effect on aspergillosis has also been reported. Probable chemical structure of HA-1-92 has also been determined. The present communication reports the hypolipidemic, hypoprostatic and other pharmacological activities of HA-1-92.

Acute toxicity in mice and rats – HA-1-92, prepared and tested in research and development laboratory was used. HA-1-92 was prepared freshly as suspension in 0.1N NaH$_2$PO$_4$ solution, adjusted to pH 7.2 with NaOH prior to experiments.

Albino wistar rats (12-14 weeks old) weighing 150-120 g and Swiss albino mice (11 weeks old) weighing 20-25 g were divided into different groups of 10 animals each. Animals were housed at 24°C under 10 hr light (electricity) and allowed to free access to food and water. Other animals, guinea pigs, rabbits and frogs were used for miscellaneous pharmacological screening as specified. Different doses of HA-1-92 were prepared as described earlier and administered to various groups of animals by different routes (ip, iv and orally). Treated animals were subjected for behavioural observation and recorded mortality after 7 days of treatment. LD$_{50}$ by different routes was calculated with 95% confidence limits by the method of Litchfield and Wilcoxon.$^4$

Hypolipidemic activity in rats and rabbits – Hypolipidemic activity was assessed by administering HA-1-92 (100 mg/kg/day) orally for 15 days to old obese rats (around 30 months old; weighing 330-360 g) and rabbits (36-38 months old; weighing around 4 kg) and analysed blood samples on day 8 and 16 day for low density lipoprotein (LDL) and high density lipoprotein (HDL) using Autozyme kit (AccurexBiomedicals, Mumbai). Serum triglycerides$^5$ and serum cholesterol levels were also determined. Prostate glands of treated rats and rabbits were removed on day 16 and their weight, size and histological changes were recorded and compared with that of untreated control obese rats and rabbits. Serum HA-1-92 concentration on day 16 was also assayed by agar plate diffusion method using £P$.variotti$ (HA-1056). Effect of HA-1-92 on blood pressure and respiration was studied using normotensive anaesthetised rat. Effect of HA-1-92 was also studied on isolated tissue preparations like heart (rabbit), rectus abdominis muscle (frog) and ileum (guinea pig) using appropriate experimental conditions$^7$.

Statistical analysis – Data was analysed statistically to find out level of significance using Student's-test.$^8$ The values at $P(<0.05)$ were considered significant.

Acute toxicity – LD$_{50}$ of HA-1-92 in mice and rats by different routes are shown in Table1. Oral administration of HA-1-92 induced anorexia, diarrhea and stomach distension. However, intra peritoneal administration of HA-1-92 elicited local irritation at the site of injection (revealed by writhing

---

*Present Address—Prof. Of Pharmacology & Biotechnology, K.M.Kundnani College of Pharmacy, Dr.R.G. Thadani Marg, Mumbai – 400 018, India.
episodes) which lasted for 2-3 hr. Mice and rats also exhibited ataxia, respiratory distress, loss of muscle tone followed by death at higher doses. Most of the animals treated with HA-1-92 died due to respiratory arrest. Low toxicity of HA-1-92 by oral route could be attributed to poor absorbance of HA-1-92 in gastro-intestinal tract and may spread over several hours.

Hypolipidemic activity—Treatment with HA-1-92 (100mg/kg/day) orally for 15 days reduced serum triglycerides, total cholesterol and LDL levels of old obese rats and rabbits significantly, which is suggestive of its hypolipidemic activity (Table 2). A significant reduction in weight of prostate gland was also observed in HA-1-92 treated obese rats and rabbits as compared to non-treated control animals (Table 2). No significant change was observed in HDL levels after 8 days of treatment. However, it was evident that there was a tendency to increase HDL levels by HA-1-92 on longer treatment (for 15 days) in rats and rabbits which may be attributed to higher serum concentration of HA-1-92 (Table 2).

Hypocholesterolemic activity of hamycin, a polyene macrolide antibiotic discovered in our laboratory has been demonstrated by using similar animal model 9,10. Plasma lipids circulate as

<table>
<thead>
<tr>
<th>Table 1—acute toxicity studies of HA-1-92.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Route</strong></td>
</tr>
<tr>
<td>Oral</td>
</tr>
<tr>
<td>Orally</td>
</tr>
<tr>
<td>iv</td>
</tr>
<tr>
<td>iv</td>
</tr>
</tbody>
</table>

*Please also see Results & Discussion section.

Hypolipidemic activity—Treatment with HA-1-92 (100mg/kg/day) orally for 15 days reduced serum triglycerides, total cholesterol and LDL levels of old obese rats and rabbits significantly, which is suggestive of its hypolipidemic activity (Table 2). A significant reduction in weight of prostate gland was also observed in HA-1-92 treated obese rats and rabbits as compared to non-treated control animals (Table 2). No significant change was observed in HDL levels after 8 days of treatment. However, it was evident that there was a tendency to increase HDL levels by HA-1-92 on longer treatment (for 15 days) in rats and rabbits which may be attributed to higher serum concentration of HA-1-92 (Table 2).

Hypocholesterolemic activity of hamycin, a polyene macrolide antibiotic discovered in our laboratory has been demonstrated by using similar animal model 9,10. Plasma lipids circulate as

<table>
<thead>
<tr>
<th>Table 2—Effect of 15 days treatment of HA-1-92 on the serum lipid level of old obese rats and rabbits</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum Lipid levels (mg%)</strong></td>
</tr>
<tr>
<td><strong>Animal</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Obese rat</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Obese rabbit</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

A—untreated control, B & C—HA-1-92 treated 8th & 16th day observations.

Figure in parentheses indicate number of animals used.
lipoproteins i.e., a mixture of protein, cholesterol, glycerides and phospholipids of which major classes are VLDL, LDL and HDL. Cholesterol which is present in atherosclerotic lesions comes from local biosynthesis. LDL-carried cholesterol largely correlates to their physical binding with cholesterol in gastrointestinal tract and also to an increased uptake and utilisation of circulating cholesterol by tissues. Other possible factor contributing to hypolipidemic activity of HA-1-92 would be its inhibitory effect on biosynthesis of cholesterol in liver like amphotericin B leading to decreased formation of plasma lipoproteins.

Reduction in body weight observed during 15 days treatment may be due to adverse effect of HA-1-92 on animals i.e., decreased food intake and abdominal swelling from gas distension in the intestine. Probable mechanism(s) of action associated in reduction of prostate gland size following HA-1-92 treatment needs to be investigated further. However, earlier reports indicate that antifungal treatment decreases serum testosterone levels and presume that the same mechanism might be playing either partly or solely for eliciting the hypoprostatic effect observed with HA-1-92 in the present study. At the same time, possibility of direct interaction of parent molecule or some metabolite thereof with organs involved is still open for study. At the same time, influence of nutritional status and well being of animal in reduction of prostate gland cannot be ruled out and needs to be evaluated.

Miscellaneous pharmacological screening—Findings of the other pharmacological experiments indicate that HA-1-92 (1 mg/kg; iv) induced very transient (4 min) and insignificant fall in blood pressure (5-7%). No alteration was observed either in pressor responses of epinephrine and nor-epinephrine or depressor responses of acehtylcholine and histamine. However, HA-1-92 did produce a mild decrease in respiratory rate for a period of 40 min. On isolated tissue preparation, e.g., smooth muscle (guinea pig ileum), skeletal muscle (frog rectus abdominis muscle), HA-1-92 (50-1000 µg/mL) did not induce contraction per se or blocked the contraction elicited by agonists like histamine or acehtylcholine. HA-1-92 up to (100 µg) failed to show any effect on heart rate, force of contraction or change in coronary flow in an isolated rabbit heart preparation.

In conclusion, HA-1-92, a new oxohexaene polyene antibiotic, showed promising hypolipidemic and hypoprostatic activity both in rat and rabbit models. Exact mechanism(s) of hypolipidemic and hypoprostatic activity of HA-1-92 needs to be established by studying in other animal models and also its effects on cholesterol LDL biosynthetic pathways.

Authors are grateful to the Director, Central Drug Research Institute, Lucknow, India for supplying lyophil CDRIL-312.

References