TOXICOLOGICAL STUDIES ON AN ANTICANCER DRUG (HESA-A) WITH MARINE ORIGIN

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SUMMARY: HESA-A, which contains biologically active compounds of marine origin, has selective toxicity against cancer cells. The present work reports the results of studies investigating the acute and sub-acute oral toxicity of this drug in mice and rats. In acute toxicity study, doses of HESA-A up to 13.7 g/kg and in sub-acute study, oral doses of 1250, 2500 and 5000 mg/kg for 30 consecutive days did not cause any morbidity or mortality. Data analysis of body weight gain, gross observations, blood biochemistry, hematology and histopathological findings did not show significant differences between control and treated groups. An oral dose of 5000 mg/kg of HESA-A can be defined as no-observed-adverse-effect-level (NOAEL) for mice and rats used under the experimental conditions.

Key Words: Anticancer, HESA-A, toxicity.

INTRODUCTION

HESA-A, which contains biologically active compounds of marine origin, is patented under Iranian authority. It has been claimed that this drug has considerable anticancer effect. Recently it has been reported that HESA-A selectively and in a concentration dependent manner inhibited the growth of cancer cells and it had no toxicity on normal cell lines (17). HESA-A is a mixture of many organic salts or complexes and also contains trace amounts of Br, Sr, Ti, Mn, Ni, Ag, Cu, Zn, Cs and Cd (17). Several authors have reviewed the toxicity of some of these elements (1,6,15,16,18,21). Taking this background into account, the studies reported here were performed to investigate the oral acute and sub-acute toxicity of HESA-A in animal models.

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MATERIALS AND METHODS

Animals

Swiss albino mice (25–30 g) and young adult Wistar rats weighing 180±20 g of both sexes were purchased from the Pasteur Institute (Tehran, Iran). All animals were housed in animal

rooms where the temperature was 22±2°C and in 12 h light-dark cycle. They were fed with regular rat chow and tap water *ad libitum*. Prior to each experiment animals were fasted overnight and allowed free access to water (8). All animal manipulations were performed according to the ethical principles for animal care and management recommended by ethical committee of Isfahan University of Medical Sciences.

Chemicals

All chemicals and solvents used throughout this investigation were of analytical grade.

Acute study

Seven groups of mice, each containing 4 animals (2 males and 2 females) were used. One group was used as control and other groups received HESA-A orally at doses of 4500, 5625, 7030, 8790, 10985 and 13730 mg/kg. Increases in doses obey a factor of 1.25. The starting dose was based on pilot experiments in which doses of HESA-A up to 4500 mg/kg did not induce any mortality. Control animals received vehicle in a volume of 10 ml/kg. Prior to experiments, animals were fasted overnight with free access to water.

Sub-acute study

Sub-acute toxicity of HESA-A was investigated in mice and rats of either sex in groups of 10. Animals with free access to food and water were orally given various sub-lethal doses of HESA-A ranging from 1250 to 5000 mg/kg once a day for 30 consecutive days. The control animals received vehicle (aqueous solution of carboxymethylcellulose 1% w/v) in a volume of 10 ml/kg. During this period, animals were observed for awareness, status of mood, motor activity, CNS excitation, posture, muscle tone, reflexes and autonomic signs each day. Also weight of rats was measured and recorded at 5 days intervals (7). At the end of the experimental period, the animals were sacrificed under ether anesthesia, following an overnight fast and blood samples were collected. Whole blood of mice and serum of rats were used for hematological and biochemical tests, respectively.

To obtain the serum, blood samples were placed at room temperature for approximately 30 minutes. Then, the tubes were centrifuged at 3000 x g for 10 mins and the supernatants were taken to perform the following determinations: serum glucose, urea, creatinine, albumin and globulin concentration and also enzyme activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH).

Hematology parameters

The hematology parameters including hemoglobin concentration, hematocrit and number of red and white blood cells and also platelets were analyzed using an H-1 automated hematology analyzer (USA).

Biochemical assays

Activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase and also serum concentration of glucose, urea, creatinine, albumin and globulin were determined using an auto analyzer (RA-1000, USA).

Autopsy and histopathology

During autopsy, all organs were observed macroscopically and selected organs or tissues including brain, heart, kidneys, liver, spleen, stomach, large and small intestine, lungs, skin and skeletal muscle were excised and then fixed in 10% buffered neutral formalin solution. Paraffin sections were prepared and stained with hematoxylin and eosin for histological examination. All slides were initially read by one pathologist and peer reviewed by the second pathologist.

Statistical analysis

Values were expressed as mean±SD. Means of treated groups were compared with those of control groups using one-way analysis of variance (ANOVA) followed by Dunnett's test (9,12). Differences were considered statistically significant if $p<0.05$.

RESULTS

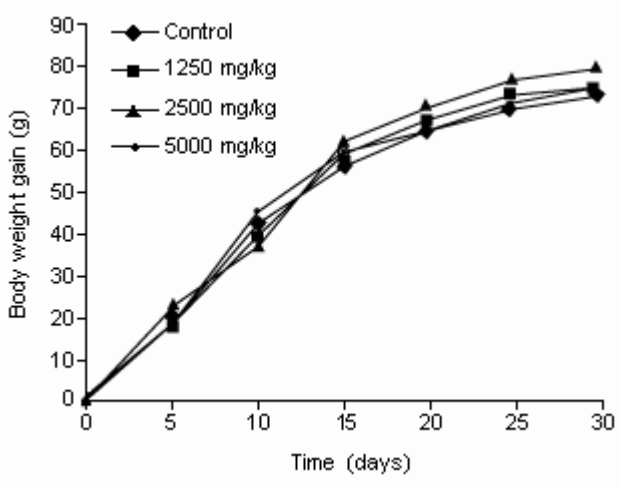
Acute toxicity

Single oral doses of HESA-A up to 13.7 g/kg could not cause any morbidity or mortality in the animals.

Sub-acute toxicity

A daily oral administration of HESA-A at doses of 1250, 2500 and 5000 mg/kg to mice and rats during 30 days failed to cause any death and did not change the animal's

Figure 1: Effect of different doses of HESA-A on body weight gain of rats. Wistar rats of both sexes in groups of 10 animals per each orally received vehicle (10 ml/kg) or different doses of HESA-A for 30 days. Data are mean of body weight gain (g).



general behavior and autonomic signs. Figure 1 shows the weight gain of control and test animals. HESA-A during the one month period of experiment could not affect the weight gain of animals. Table 1 depicts the drug effect on hematological parameters of mice. As it is observed, HESA-A even at the highest dose used, did not alter hemoglobin concentration, hematocrit and the number of red and white blood cells as well as the number of platelets. Table 2 shows the effect of HESA-A on biochemical parameters of rats. Serum concentration of glucose, urea, creatinine, albumin and globulin as well as the activity of marker enzymes (AST, ALT, ALP, LDH) in HESA-A treated groups were not significantly different from control values. Histologic examination of the main organs did not reveal morphological changes of tissues that could be clearly related to the treatment with HESA-A. Only a slight hepatic infiltration occurred at a dose of 5000 mg/kg.

DISCUSSION

According to the results of this study, it seems that HESA-A is a safe drug. The drug even at high doses did not affect the general behavior or autonomic signs of animals. The weight gain in HESA-A treated animals was not different from control animals. Many anticancer drugs damage the epithelial cells of the digestive tract, cause anorexia, nausea and vomiting, malabsorption and these effects cause weight reduction (5, 20). Since HESA-A did not influence the weight gain, it is unlikely to produce toxic changes above side effects on alimentary canal.

Anticancer drugs such as aminoglutethimide, azathioprin, cyclophosphamide and methotredate cause hepatotoxicity, some drugs including cisplatin and cyclosporin produce nephrotoxicity (3,10,11,14,19). Transaminases (AST, ALT) and alkaline phosphatase are good indices of liver and kidney damage, respectively (13). The drug did not induce any damage to liver and kidney which could be inferred from normal activity of these enzymes. Since HESA-A did not alter the blood concentrations of urea and creatinine, this again confirms that the drug is not nephrotoxic.

LDH activity of HESA-A treated groups were not significantly different from control group. LDH has 5 isoenzymes. The isoenzymes are composed of 2 different types of subunits, called M and H, that are combined randomly with each other in a tetrameric structure. The

Table 1: Hemotological profile of mice fed different doses of HESA-A for 30 days.

Groups	Dose (mg/kg)	Hb (g/dl)	HCT (%)	RBC Cells X 10^6/mm^3	WBC Cells X 10^3/mm^3	PLT Cells X 10^5/mm^3
Control	0	11.2 ± 0.5	41.5 ± 1.2	5.8 ± 0.6	4.1 ± 0.4	9.1 ± 0.3
HESA-A	1250	11.5 ± 0.3	42.5 ± 1.8	5.7 ± 0.5	4.2 ± 0.4	9.0 ± 0.6
	2500	11.3 ± 0.6	42.0 ± 0.9	6.1 ± 0.8	4.0 ± 0.3	8.7 ± 0.4
	5000	11.5 ± 0.6	42.2 ± 1.3	5.9 ± 0.6	4.2 ± 0.6	9.0 ± 0.7

Each value is mean±SD of 10 mice in each group. Hb: Hemoglobin, HCT: hematocric, RBC: red bood cell, WBC: white blood cell, PLT: platelet

Table 2: Effect of HESA-A on biochemical parameters of rats.

Groups	Dose (mg/kg)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)	Globulin (g/dl)	AST (IU/ml)	ALT (IU/ml)	ALP (IU/ml)	LDH (IU/ml)
Control	0	83±3	38±3	0.41±0.05	4.15±0.11	1.95±0.08	89.7±8.6	26.0±2.9	39.5±4.3	1601±108
HESA-A	1250	79±4	42±5	0.39±0.06	4.20±0.18	1.99±0.11	95.2±10.2	27.4±5.6	37.2±5.8	1546±154
	2500	81±4	39±5	0.46±0.07	4.08±0.21	2.02±0.06	103.3±22.5	31.0±6.1	37.1±4.9	1485±211
	5000	78±5	41±3	0.40±0.04	4.22±0.13	1.89±0.14	94.2±14.1	26.3±2.8	38.6±5.7	1392±290

Values are mean±SD of 10 animals in each group. AST: aspartate transaminase, ALT: alanine transaminase, ALP: alkaline phosphatase, LDH: lactate dehydrogenase.

five major isoenzymes have the compositions M_4, M_3H, M_2H_2, MH_3, and H_4 with M subunits predominant in skeletal muscle and the liver and H subunits predominant in the heart (2, 4). Although the activity of different isoenzymes of LDH was not determined in our study, however, since the total activity of LDH was not altered by HESA-A, it seems that the drug did not damage the skeletal muscle and heart cells.

Bone marrow depression, or myelosuppression is also common to the majority of antineoplastic agents and is probably the single most important dose-limiting adverse effect (5). HESA-A did not affect the number of blood cells as well as Hb concentration and hematocrit and therefore it is unlikely to produce bone morrow toxicity.

In conclusion, since in sub-acute study, an oral dose of 5000 mg/kg of HESA-A administered for 30 days did not induce any biochemical, hematological and histopathological sign of toxicity, it can be defined as no-observed-adverse-effect level (NOAEL) for mice and rats used under the experimental conditions. However, it should be emphasized that this NOAEL was derived from only a sub-acute study. For a more reliable safety evaluation performed on the basis of the acceptable daily intake concept data on the long-term toxicity, reproductive toxicity, genotoxicity and carcinogenicity of HESA-A are also required.

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