Intravenous human umbilical cord blood improves electrophysiological and metabolic properties in ISO induced myocardial necrosis in rats

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Rats treated with isoproterenol (ISO, 85 mg/kg, sc, twice at an interval of 24 h) showed a significant increase in heart rate, mean arterial blood pressure, pressure rate index, ST elevation on ECG, and a significant increase in the levels of cardiac marker enzymes- lactate dehydrogenase, and creatine kinase in serum and a significant reduction in superoxide dismutase, and catalase and increase in thiobarbituric acid reactive substance activity in heart tissue. Treatment with Human umbilical cord blood (hUCBC; 500 and 1000 µL, iv, via the tail vein; 2 h after the second dose of ISO) significantly restored back to normal levels and showed a lesser degree of cellular infiltration and infarct size in histopathological and planimetry studies respectively. Thus, hUCBC ameliorates cardiotoxic effects of isoproterenol and may be of value in the treatment of myocardial infarction.

Keywords: Antioxidant enzymes, Cardiac marker enzymes, ECG, hUCBC, Isoproterenol

Human umbilical cord blood (hUCBC) is a promising source for regeneration therapy in humans. Recently it was shown that cord blood (CB) was a source of adult stem and progenitor cells and is one potential source for transplantation1,2. The potential therapeutic benefits of hUCBC for the treatment of injuries, diseases, and neurodegeneration are becoming increasingly recognized. The infusion of cord blood cells in various animal models, such as ischemia/stroke, traumatic brain injury, myocardial infarction, Parkinson’s disease, and amyotropic lateral sclerosis has shown promising results. The mesenchymal cells could differentiate into a number of cells including cardiomyocytes when injected into normal or acutely injured myocardium3. It has been reported that stem cells differentiate in the heart of rodent models as well as improve heart function, caused by cardiac injury4. hUCB cells do not raise ethical issues, have weak immunogenicity, and are proven to be safe in the treatment of paediatric diseases5. Cell transplantation is now considered as an alternative therapy for treating patients with end-stage organ failure. Fetal cardiomyocytes, skeletal myoblasts, immortalized cell lines, fibroblasts, smooth muscle cells and haematopoietic stem cells have been transplanted into host myocardium for improved cardiac function5. Myocardial infarction (MI) is one of the main causes related to sudden death in the world. It is hypothesized that intravenously infused hUCBC would reach to the zone of injury and attenuate infarction parameters. Since damage to myocardium caused by isoproterenol results in necrosis which to a certain extent simulates the condition of myocardial infarction in humans, but without any effect on the blood supply to the myocardium, the cardioprotective benefits of human cord blood cells have been explored in isoproterenol–induced myocardial necrosis in rats.

Materials and Methods

Experimental animals—Male adult albino Wistar rats, weighing 150-200 g were obtained from Bharat Serum and Vaccines Ltd. Thane, Mumbai, India. They were housed in polypropylene cages lined with husk, renewed every 48 h under 12:12 h light dark cycle and maintained at 25 ± 2 °C. They were fed with commercial pellet rat chow and given water ad libitum. The experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals

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Tracheostomy was performed.

Drugs and chemicals—Isoproterenol hydrochloride (ISO), was purchased from Sigma-Aldrich, Mumbai. All other chemicals used in the study were of analytical grade. All drug solutions were freshly prepared in saline before each experiment. Metoprolol hydrochloride (Betaloc, Astra Zeneca, Mumbai) was purchased locally. Human umbilical cord blood cells were obtained from Government Hospitals and Private Maternity Homes of Nashik District and maintained under cold storage conditions (0-5 °C). Freely given informed consent was obtained from mothers from whom the cord blood was collected. The protocol for the study was approved by institutional Review Board and experiments were carried out as per CPCSEA guidelines. Serum were obtained from Government Hospitals and approved by the institutional Animal Ethical Committee.

Experimental design—ISO (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 h for 2 days to induce experimental MI. Animals were divided into following 6 groups of 5 each: Gr. I: Control (received saline); Gr. II: ISO (85 mg/kg, sc) twice at an interval of 24 h; Group III: ISO (85mg/kg, sc) twice at an interval of 24 h along with metoprolol (5 mg/kg, iv 2 h after second dose of ISO); Gr. IV: ISO (85mg/kg, sc) twice at an interval of 24 h along with hUCBC (500 μL via tail vein after 2 h of second dose of ISO); Gr. V: ISO (85mg/kg, sc) twice at an interval of 24 h along with hUCBC (1000 μL via tail vein after 2 h of second dose of ISO); Gr. VI: hUCBC (500 μL) via tail vein. After 3 h following the last dose of ISO, heart rate, ECG, mean anterial pood pressure (MABP) was recorded using Powerlab4SP (ADInstrument, Australia). The sum of the infarcted area of an individual slice was averaged on the basis of their weight to calculate the total ventricular ischemia for each heart.

Histopathological examination—The hearts were excised and immediately fixed in 10% buffered formalin. The ventricular mass was sectioned from the apex to the base of the heart, which was embedded in paraffin after being dehydrated in alcohol and
subsequently cleared with xylene. Serial histological sections (5 µm thick) were obtained from the paraffin blocks and stained with hematoxylin and eosin. The sections were examined under light microscope and photomicrographs were taken.

Results

Effect of hUCBC on heart rate, MABP and pressure rate index—The heart rate, MABP and PRI in vehicle treated animals was recorded as 285.4 ± 19.5 beats/min, 89.6 ± 3.1 mmHg and 25.65 ± 1.97 mm Hg min⁻¹ respectively. ISO treated rats showed a significant (P<0.05) elevation in heart rate, MABP and PRI. Treatment with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) in ISO treated animals restored the heart rate, MABP and PRI to near normal values (Table 1).

Effect of hUCBC on ECG—The R-R interval and ST height in vehicle treated animals was recorded as 0.214 ± 0.014 msec and -0.050 ± 0.012 mm respectively. ISO treated rats showed a significant (P<0.05) changes in ECG parameters. Treatment with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) in ISO treated animals restored the ECG changes (Table 2).

Effect of hUCBC on cardiac marker enzymes—The serum LDH and CK-MB concentrations in vehicle treated rats were 11.31±1.70 and 4.27±0.68 IU/L. Rats treated with ISO, showed a significant (P<0.05) increase in the activities of these enzymes in serum. Rats treated with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) in ISO pre-treated rats significantly (P<0.05) reduced the activities of enzymes as compared to ISO-treated rats (Fig. 1).

Effect of hUCBC on antioxidant enzymes—The activities of antioxidant enzymes- SOD, CAT and TBARS. SOD and CAT activities were decreased significantly (P<0.05) in ISO treated rats when compared to those of control rats (Table 2). TBARS level was significantly increased in ISO treated rats when compared to those of control rats. The activities of antioxidant enzymes were maintained at near normal levels in animals treated with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) in ISO pre-treated rats.

Effect of hUCBC on TTC staining, planimetry and histopathology—The heart tissues of control group were stained brick red (dark region) with TTC, an indicator of mitochondrial respiration. The unstained region is an indicator of total necrosis as seen in ISO

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<th>Table 1—Effect of hUCBC (500 and 1000 µL, iv, via tail vein) on various cardiac parameters in isoproterenol (85 mg/kg, sc) induced MI in male rats [Values are mean ± SE from 5 observations each]</th>
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<th>Table 2—Effect of hUCBC (500 and 1000 µL, iv, via tail vein) on biomarkers of oxidative stress in isoproterenol (85 mg/kg, sc) induced MI in male rats [Values are mean ± SE from 5 observations each]</th>
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treated group. The sections of heart tissues treated with hUCBC in ISO group showed a lesser degree of unstained region compared to ISO treated group (Fig. 2). The control animal does not exhibit infarct size. The ISO administered group significantly increased the infarct size as compared to control animals. Rats post-treated with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) via tail vein after 2 h of second dose of ISO (85 mg/kg, sc) treatment showed a significant (P<0.05) decrease in infarct size as compared to ISO treated group (Fig. 3). In histopathological examination, normal architecture was observed in control animals whereas animals treated with ISO showed thrombus formation, contraction band necrosis and inflammation. Animals treated with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) in ISO pre-treated animals revealed much less intensity of the above changes (Fig. 4).

Discussion

Isoproterenol induced MI is a well known model to study the beneficial effects of many drugs and cardiac function. Myocardial necrosis is produced by ISO in large dose. Oxidation of hydroxyl groups in catecholamines thereby leading to the conversion into quinones and the subsequent formation of adrenochromes are responsible for the toxic effects of ISO. Highly toxic oxygen-derived free radicals are generated which are toxic to extra- and intracellular enzymes and proteins.

The main criterion generally used for the definite diagnosis of MI is the changing pattern of ECG-abnormalities. Significant alterations of ECG patterns were observed in ISO-induced rats when compared with normal rats. The characteristic findings were the elevation of ST segment and reduction of R-R interval, which are the indicative signs of ischemia. It is well known that the ST elevation correlates well with the leak of CK from the myocardium and the degree of damage observed histologically. Raised serum levels of cardiac specific markers of acute myocardial infarction—CK-

![Fig. 1—Effect of hUCBC (500 and 1000 µL, iv, via tail vein) on cardiac marker enzymes in isoproterenol (85 mg/kg, sc) induced MI in male rats. [Values are mean ± SE from 5 observations each. P values: <0.05; against control group, ISO group, one way ANOVA followed by Dunnett’s test. Gr. 1: Control (vehicle treated), Gr. 2: ISO (85 mg/kg, sc, at interval of 24 h for 2 days), Gr. 3: Metoprolol (5 mg/kg, iv, via tail vein) + ISO (85 mg/kg, sc, at an interval of 24 h), Gr. 4: hUCBC (500 µL, iv, via tail vein) + ISO (85 mg/kg, sc, at an interval of 24 h), Gr. 5: hUCBC (1000 µL, iv, via tail vein) + ISO (85 mg/kg, sc at an interval of 24 h), Gr. 6: hUCBC (500 µL, iv, via tail vein) ISO = Isoproterenol, hUCBC = human umbilical cord blood cell]
MB or LDH, is an indication of myocardial tissue injury. The consecutive loss of cell membrane function in the regional ischemic myocardium might be characterized by ST-segment elevation, increase of ventricular excitability, conduction disturbances, and tachycardia. Pressure rate index was calculated as an index of oxygen demand in all the groups. ISO treatment significantly increased PRI and the heart rate which is in congruence with Kela et al. In the present study metoprolol, a beta antagonist was used as a standard drug. Pretreatment with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) showed a protective effect against ISO-induced alteration in the heart rate, PRI, ECG patterns and protected the cell membrane damage as evident by the gross morphologic and histopathologic changes.

Significant elevation was noticed in the levels of diagnostic marker enzymes (LDH and CK) in the serum of ISO group as compared to other groups, which is in the line with the earlier reports. hUCBC treatment restores the levels of cardiac marker enzyme to near normal. This indicates the efficacy of hUCBC in reducing the severity of ISO induced necrotic damage to the myocardial membrane.

Free radical scavenging enzymes such as catalase, superoxide dismustase are the first line cellular defense enzymes against oxidative injury, decomposing O$_2$ and H$_2$O$_2$ before their interaction to form the more reactive hydroxyl radical (OH$^-$). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. Malondialdehyde formed by the breakdown of lipid peroxides is often used to quantify the extent of lipid peroxidation. The SOD and CAT activities were decreased; whereas TBARS levels were increased in ISO treated group as compared to the other groups. During myocardial infarction, superoxide radicals generated at the site of damage modulates SOD and CAT resulting in the loss of activity and thus damage myocardium. hUCBC treatment increased the activity of SOD, CAT and

Fig. 4—Histopathological examination (10X) of rat heart in various treated groups [A: Control (vehicle treated), B: ISO (85 mg/kg, sc) twice at an interval of 24 h, C: ISO (85 mg/kg, sc) twice at an interval of 24 h along with metoprolol (5 mg/kg, iv 2 h after second dose of ISO), D: ISO (85 mg/kg, sc) twice at an interval of 24 h along with hUCBC (500 µL iv via tail vein after 2 h of second dose of ISO), E: ISO (85 mg/kg, sc) twice at an interval of 24 h along with hUCBC (1000 µL, iv) via tail vein, Section of heart from Group A showing normal cardiac architecture. Section of the heart from Group B reveals structure disruption, increased eosinophilia, loss of nuclei, degenerative changes and inflammation. Sections of heart from group D shows lesser loss of nuclei and eosinophilia as compared to Group B, whereas sections of heart from groups C, E and F showed near normal cardiac architecture as compared to group B.
decreased TBARS levels indicating increased removal of superoxide radicals and decreased malondialdehyde levels, thereby reducing myocardial damage.

In presence of intact mitochondrial dehydrogenase enzyme system, TTC forms a red formazan precipitate with LDH of the viable myocardial tissue, whereas areas of necrosis lack mitochondrial dehydrogenase activity and do not stain. Consequently, areas not stained with TTC correspond to areas of total necrosis. In the present study, myocardial necrosis by direct staining using TTC dye, confirmed that in ISO administered groups there was a significant leakage of LDH as compared to control. Treatment with metoprolol (5 mg/kg, iv) and hUCBC (500 and 1000 µL, iv) in ISO treated animals showed a lesser degree of unstained region and a lesser infarct size as shown by planimetry studies. On histopathological examination, ISO treated group, demonstrated loss of nuclei, structure disruption, degeneration and inflammation. Treatment with hUCBC reversed these changes.

It has been shown that hUCBC successfully reverses the pathological effects of ISO-induced cardiac injury, but the study lacks the mechanistic details on why and how do the hUCB cells migrate towards ISO challenged hearts and produce the desired changes. It is concluded that hUCBC has a potential to inhibit the cardiotoxic effects induced by ISO and possesses a significant therapeutic value in the treatment of myocardial necrosis.

Conflict of interest
The authors have no conflict of interest.

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