ALLOGENEIC LIVER CELL SUSPENSION IN THE TREATMENT OF HEPATIC FAILURE

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The possibility of using isolated hepatocytes for the treatment of liver diseases was studied in animals. The optimal dose of a cell suspension for intravascular, intraperitoneal, intrapleural, and subcutaneous injection was established and the response of experimental animals to this biological substrate was studied. The effectiveness of isolated hepatocytes in the treatment of hepatic failure was demonstrated and the various methods of injection of the cell suspension were compared.

KEY WORDS: hepatocyte; hepatic cell failure; peritoneal dialysis; allogeneic and xenogeneic suspension of hepatocytes; intact cell.

All the methods currently used to treat hepatic failure can be subdivided into conservative and surgical [2-8]. However, these methods do not give the necessary therapeutic effect and are aimed mainly at removing metabolic products or reducing their blood concentration, with the result that the lost functions of the affected liver are only partially replaced. Since 1970 the authors have been engaged in experimental research to develop and study methods of active treatment of hepatic failure, using an allogeneic cell suspension for this purpose.

A liver cell was first isolated in 1943 [16]. Existing methods of isolating hepatocytes now can be subdivided into four groups: mechanical [9, 10], dissociating [12, 13, 15], enzymic [14], and combined [11, 15]. According to some workers, regardless of the method used to obtain the isolated hepatocyte, it is viable and functioning cell [1, 10, 13, 14, 16].

EXPERIMENTAL METHOD

Experiments were carried out on 540 male albino rats weighing 100-150 g. A combined method, developed by Archakov et al. [1], was used to isolate the cells (Fig. 1). The cells in the medium were counted in a Goryaev chamber. The mean number of liver cells in 1 ml suspension reached 2×10^7. The viability of the cells was determined by morphological [5, 13, 14, 16] and biochemical methods [1, 10, 15].

Four series of experiments were carried out. In the experiments of series I (150 rats) biologically tolerable doses of a cell suspension were determined in healthy rats. The animals of this series of experiments

TABLE 1. Survival Rate of Animals Depending on Method and Dose of Cell Suspension Injected

Method of injection	Dose of cell suspension, ml					
	2,5	2,0	1,5	1,0	0,5	0,25
Intravascular	3	4	4	5	5	5
Intraperitoneal	4	4	5	5	5	5
Intrapleural	3	4	5	5	5	5
Subcutaneous	4	5	5	5	5	5
Intravascular (killed cells)	—	—	1	2	3	5

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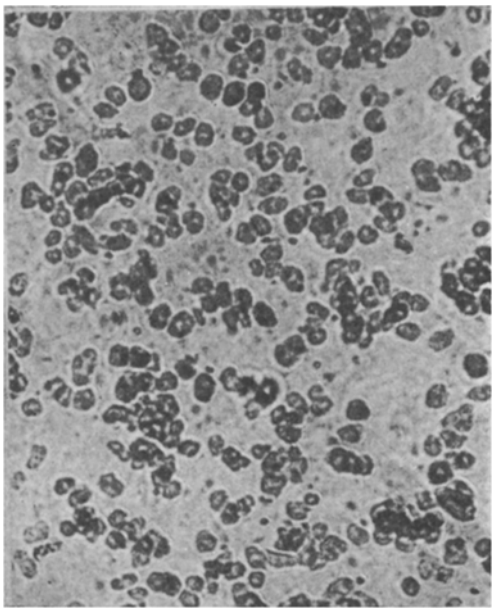

Fig. 1. Isolated rat liver cells obtained by the combined method. Stained with 0.2% methylene blue solution; 48×.

TABLE 2. Results of Treatment of Animals with Hepatic Failure by Allogeneic Liver Cell Suspension

Series of experiments	Method of injection of cell suspension	Dose of cell suspension, ml	Group of animals	Number of animals	Number of animals which died	Per cent of animals which died	Length of survival of animals		P, %
							M	±m	
II – ligation of hepatic artery	i/v	1,0	E	22	10	45,5	13,7	0,97	0,3
			C	22	16	76,3	7,9	1,7	
		0,5	E	14	7	50,0	16,2	1,3	0,1
			C	14	12	85,7	6,6	2,3	
		0,25	E	10	4	40	13,8	1,6	15
			C	10	6	60	9,4	2,7	
	i/p	1,0	E	10	6	60	11,9	1,8	9
			C	10	8	80	7,0	2,1	
		0,5	E	30	12	40	16,2	1,0	0,1
			C	30	24	80	6,4	1,2	
III – poisoning with CCl_4	i/p	1,0	E	34	18	52,7	19,6	1,2	0,3
			C	34	27	79,4	12,8	1,8	
		0,5	E	30	9	30	16,2	1,3	0,1
			C	30	22	73,6	9,6	1,5	
IV – stenosis of portal vein	i/v	1,0	E	15	4	26,6	26,8	1,5	0,4
			C	15	10	66,7	16,2	3,1	
	i/p	1,0	C	30	7	26,6	18,9	0,4	0,1
			E	30	18	60,0	12,3	1,5	

Legend. E) Experimental; C) control; i/v) intravascular; i/p) intraperitoneal.

were divided into several groups, with 5 rats in each group, depending on the dose [2.5, 2.0, 1.5, 1.0, 0.5, and 0.25 ml) and method of injection of the cell suspension (intravascular, intrapleural, intraperitoneal, subcutaneous).

In the experiments of series II, III, and IV (390 rats) a model of hepatic failure was first produced. In series II the model was created by ligating the hepatic artery. Depending on the method of injection of the cell

suspension the animals of this series were divided into four groups (172 rats altogether). Each group, depending on the dose, was subdivided into 2 or 3 subgroups. In series III the animals were poisoned with CCl_4. These experiments were carried out on 128 rats. The cell suspension in different doses was injected intraperitoneally. In the experiments of series IV stenosis of the portal vein was produced. These animals were divided into three groups, and in each group only one dose of cell suspension was given. In each series of experiments the animals with experimental hepatic failure were divided into two groups: experimental and control. The rats of the experimental group received an injection of cell suspension whereas the control animals received the same dose of physiological saline.

Observations were maintained for 15-17 days on the animals (the color of the skin, the activity of the animals), their survival rate was noted, and biochemical (ammonia, bilirubin, and urea concentrations) and morphological changes in the liver were studied. The results were subjected to statistical analysis (E. V. Montsevichyute-Éringene, 1964).

EXPERIMENTAL RESULTS

The morphological and biochemical control observations on the isolated hepatocytes revealed that the cells were viable and functionally active, for they preserved their morphological structure, cell membrane, intracellular inclusions, and biochemical functions, in particular, their ability to synthesize proteins, urea, and so on.

The results of the experiments of series I, given in Table 1, show that by the intravascular method of injection of the living cell suspension doses of 1.0, 0.5, and 0.25 ml/100 g body weight were tolerated. The rats survived even after repeated injections of that dose. When higher doses were used sometimes the animals died during the first week.

By intraperitoneal and intrapleural injection the optimal dose was 1.5 ml. This dose of cell suspension can also be regarded as permissible by subcutaneous injection, although in that case a dose of 2 ml could also be given. A different picture was observed when a suspension of killed cells was given by the intravascular method. In these cases 0.5 ml of the cell suspension, injected once or twice, caused death of the animals.

In the experiments of series II, III, and IV the animals developed clinical, biochemical, and morphological changes characteristic of hepatic failure. The rats were apathetic, drowsy, adynamic, and aggressive, and their appetitie virtually disappeared. Biochemical tests on the rats' blood showed a sharply increased level of ammonia (1.5-3.5 mg %), bilirubin (2.0-4.5 mg %), and urea (50-90 mg %). The morphological picture was characterized by a varied degree of dystrophy of the hepatocytes, edema of the parenchyma, disturbance of the orderly structure of the hepatic trabeculae in the center of the lobules, and the appearance of foci of necrosis.

In the animals in which the hepatic artery was ligated and in those poisoned with CCl_4 acute hepatic failure developed and reached its greatest degree of severity on the 4th-5th day. In the rats with stenosis of the portal vein the disease was first manifested on the 14th-15th day. Accordingly, animals with an acute and a subacute course of the hepatocellular changes could be distinguished.

The investigations showed that in those groups of experiments in which a model of acute hepatic failure was produced better results were obtained by the use of a cell suspension in a dose of 0.5 ml/100 g body weight (Table 2). The doses of the cell suspension established in intact animals and the doses used for the treatment of animals with experimental hepatic failure thus did not coincide. In the treatment of acute forms of hepatic failure the dose of the cell suspension was 0.25-0.5 ml and in the treatment of subacute forms 1.0 ml by the various methods of injection. It also follows from Table 2 that in groups in which the cell suspension was used for treatment the mortality among the animals was significantly lower and their mean survival period was significantly longer than in rats of the control group. It was accordingly concluded that the cell suspension, if injected into the affected animals by the intravascular or intraperitoneal routes, is incorporated into metabolic and detoxicating processes, replaces the function of the damaged organ, improves the animals' state, reverses the biochemical changes, reduces destruction in the liver, and creates the conditions for more rapid reparative processes in the liver itself.

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CONTRACTILE RESPONSE OF THE MYOCARDIUM OF CARDIAC PATIENTS TO CHEMICAL SCARIFICATION OF THE CELL MEMBRANE

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Strips of myocardium from the auricles of the hearts of patients with mitral stenosis (MS) and patients with cardiac septal defects (CSD) were treated with a solution of EDTA (3 mM) to increase the permeability of the cell membrane (scarification). In a 3 mM solution of ethylenehexaminetetraacetic acid (EHTA), against the background of increased permeability of the membrane to the Ca-EHTA complex, whereby the Ca^{2+} concentration in the myofibrils can be regulated between 10^{-9} and 10^{-4} M, a mechanical response of the contractile proteins to a change in Ca^{2+} concentration was recorded. Despite identical threshold concentrations ($5 \cdot 10^{-8}$ M) and saturation concentrations (10^{-4} M) of Ca^{2+}, strips from patients with MS were found to develop a maximal force per unit cross section of the strip only half as high as preparations from patients with CSD, which suggests a probable lesion of the contractile proteins in the hearts of patients with MS. The ratio between the amplitudes of contraction under conditions of complete calcium activation of the contractile proteins and a single isometric contraction for preparations obtained from patients with MS was 8-10 and from patients with CSD 4-5. It is suggested that this is the result of more profound changes in the apparatus of electromechanical coupling of the myocardium of patients with MS.

KEY WORDS: heart failure; calcium ions; contractile proteins.

Comparison of the parameters of isometric contractions of the mycodardium of the atrial auricles of patients with mitral stenosis (MS) and cardiac septal defects (CSD) reveals certain significant differences. On average the time taken to reach the maximum of the isometric contractions has been found to be appreciably longer in the myocardium of patients with MS than in the myocardium of patients with CSD. In MS, moreover, the normal response of the myocardium to an increase in the frequency of stimulation is modified much more often than in CSD, and this is reflected in the total or partial suppression of the Bowditch phenomenon [1, 3].

In view of data indicating a disturbance of the function of the sarcoplasmic reticulum (SR) in cardiac failure [2] it has been concluded that the severer disturbances of the contractile function of the myocardium

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