Glucural (Amigluracyl) Improves Survival of Isolated Rat Hepatocytes in Suspension

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Nonsteroid anti-inflammatory drug glucural (water-soluble N-methyl-D-glucosamine complex with 6-methyluracyl) improves survival of isolated rat hepatocytes stored in suspension. This effect of glucural is presumably explained by its membranotropism.

Key Words: *hepatocyte; glucural; cytoprotective effect*

Isolated hepatocytes are widely used in basic studies and practical medicine. Fetal and adult hepatocytes are used for transplantation in liver diseases [1,4]. The creation of hepatocyte suspension or primary culture-based means for replacement of damaged liver is in progress [2,3]. Hepatocyte capacity to detoxification of exogenous xenobiotics is used for creation of test systems in pharmacology [5]. The maintenance of functional activity of hepatocytes is one of the most intricate problems because storage of cell suspension is fraught with loss of metabolic characteristics of the cells and the hepatocyte culture has to be maintained for a rather long time. The use of membranotropic cytoprotectors is a promising method for prolonging lifespan of isolated hepatocytes. Our preliminary studies have shown anti-inflammatory and cytoprotective effects of glucural (amigluracyl), a water-soluble complex of 2,4-dihydroxy-6-methylpyrimidine (6-methyluracyl) with N-methyl-D-glucosamine [6].

We studied the possibility of using glucural for maintaining viability of hepatocytes during storage after their isolation.

MATERIALS AND METHODS

Rat hepatocytes were isolated by Seglen's method [7]. Hepatocyte viability was evaluated by trypan blue, acridine orange, and ethidium bromide staining and by the MTT test (at λ=570-630 nm).

Functional activity of isolated hepatocytes was evaluated by the intensity of endogenous cell respiration using adapted polarographic method [7]. The rate of oxygen consumption by hepatocytes was directly proportional to the number of viable cells in suspension and inversely proportional to the number of cells stained with trypan blue (cells with damaged membranes; Fig. 1). By extrapolation of the curve presented in Figure 1 we calculated that the endogenous respiration rate (on the proper substrata) for hepatocytes with damaged membranes was 1.9±0.1 nmol $O_2/10^6$ cell/min, while respiration rate of 100% viable hepatocytes was 20.9±0.5 nmol $O_2/10^6$ cell/min.

The significance of differences between the means was evaluated by Student's *t* test.

RESULTS

The effects of glucural were studied at different stages of hepatocyte isolation and culturing. The presence of glucural in concentrations of 100-500 μg/ml in the medium at the stage of enzymatic treatment reduced tissue dissociation and decreased the yield of hepatocytes. The positive effect of glucural was observed at the stage of centrifugation. The drug in concentrations of 100-200 μg/ml reduced hepatocyte adhesion and facilitates their resuspending.

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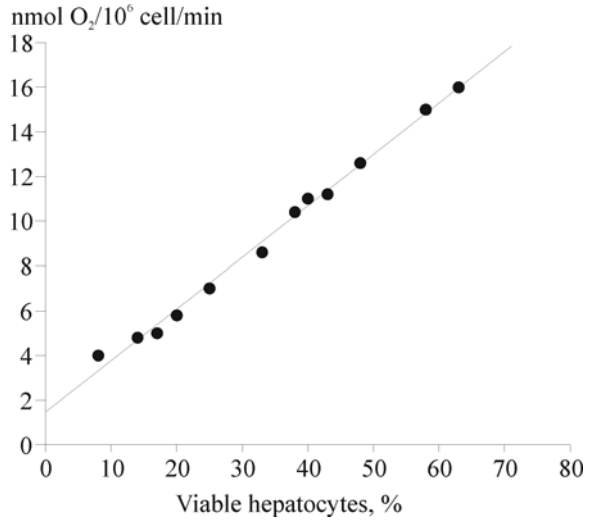

Fig. 1. Relationship between endogenous respiration rates of normal rat hepatocytes and the count of viable cells in suspension.

The effects of glucural on hepatocyte viability during culturing were studied by adding the drug into culture medium in concentrations of 50-500 μg/ml. Glucural reduced the number of hepatocytes adhering to plastic by 30% and deteriorated their spreading. The number of viable cells after 24-h culturing (MTT test) was 30-50% higher in medium without glucural than in the presence of its different concentrations; this can be explained by reduction of hepatocyte adhesion. This hypothesis was verified by culturing hepatocytes on slides coated with adhesion proteins. Evaluation of hepatocyte viability 24 h after inoculation by MTT test confirmed that the count of surviving cells in the presence of glucural was 2-fold lower than without it. Application of proteins promoting cell adhesion to the plastic did not increase the proportion of adherent hepatocytes. Fibronectin and gelatin sublayers and glucural doses of 25-200 μg/ml were tested. The counts of hepatocytes adhering to the sublayer in the presence of glucural in culture medium were 2-fold lower than in the medium without glucural in all variants of the experiment. Similar data were obtained after preincubation of cells with glucural and its subsequent removal from the medium. Morphological and functional differences between hepatocytes incubated with and without the drug were leveled after 3 days of culturing, and later the hepatocyte cultures were virtually identical.

Hepatocytes intended for transplantation are usually stored at low temperature (4°C) in suspension with a concentration of $0.5\text{-}1.0\times10^6$ cell/ml [1]. The cells remain viable about 24 h under these conditions. Trypan blue and acridine orange staining and MTT test found only 10-20% viable cells in the suspension after 24 h in our experiments. In order to evaluate the effect of glucural on hepatocyte survival during cell suspension storage, the drug was added to the medium in concentrations of 25-500 μg/ml. The presence of glucural in the medium increased the percentage of viable cells. The intensity of endogenous respiration of hepatocytes directly after isolation was 11.5 nmol $O_2/10^6$ cell/min. After 3 h of storage at 4°C the rate of O_2 consumption in hepatocyte suspension with 500 μg/ml glucural was 3.6 nmol $O_2/10^6$ cell/min *vs.* 2.1 nmol $O_2/10^6$ cell/min in medium without glucural. Evaluation of suspension viability by MTT showed that staining intensity proportional to the count of viable cells was 92.6±2.4 without glucural and 126.0±4.1 with glucural. More distinct protective effect of the drug was observed after 24 h of storage in the refrigerator, when the count of viable hepatocytes in the medium with glucural was 2-fold higher than in medium with-

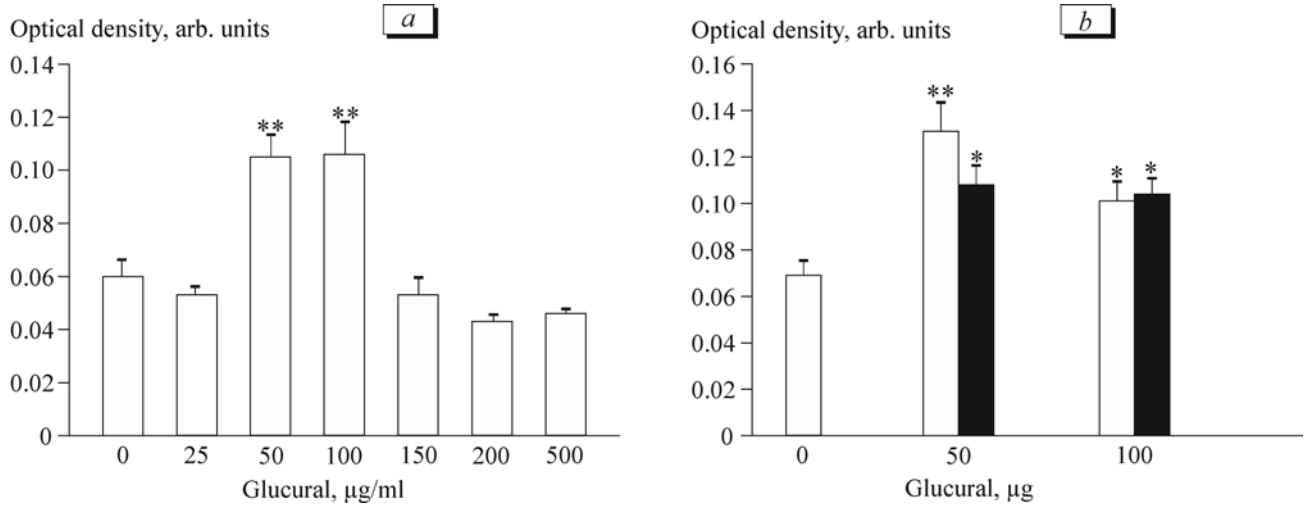

Fig. 2. Counts of viable hepatocytes after 24 h of storage at 4°C (results of MTT test) depend on glucural dose (*a*) and incubation conditions (*b*). *b*: light bars: glucural in medium; dark bars: glucural added for 2 h and washed away. *p<0.05, **p<0.01 compared to samples without glucural (0).

out it. The best results were observed at drug concentrations of 50-100 μg/ml medium (Fig. 2, *a*).

In order to clear out the mechanism of glucural interactions with hepatocytes, the cells were incubated with glucural for 2 h after which they were washed twice in medium without the drug and tested after 24 h. The cytoprotective effect of the drug persisted after its removal from culture medium (Fig. 2, *b*). These data indicate that the mechanism of the cytoprotective effect of glucural is realized at the level of hepatocyte membranes.

Hence, glucural can be used for storage of hepatocyte suspension intended for transplantation or use in bioreactors (artificial liver), when the cells should remain viable.

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