METHODS

Regression Analysis of Relationship between Liver Weight and Body Weight after Partial Hepatectomy in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 141, No. 2, pp. 234-236, February, 2006
Original article submitted September 21, 2005

A method for evaluation of the initial weight of the liver is developed for calculating the regeneration coefficient after partial hepatectomy on the basis of linear regression analysis. Experiments on rats showed that the model reflecting the relationship between liver weight and body weight for calculation of regeneration coefficient after partial resection of the liver expressed by the equation $P_0=0.033\times M$ can be used for screening of potential hepatoprotectors for quantitative evaluation of the regeneration processes.

Key Words: *partial hepatectomy; regeneration coefficient; regression analysis*

High capacity of the liver to reparation of necrotic foci after acute toxic injuries and to induction of proliferation in the remaining liver fragment after partial resection is well known [5,6,8-10]. However, in order to eliminate insufficient functional activity developing as a result of these pathological processes, stimulation of natural regeneration of the liver is needed [1]. The search for new drugs for combined therapy for normalization of functional activity and stimulation of reparative regeneration of the liver is an important problem. Screening of new drugs for evaluation of potential stimulators of regeneration in the liver parenchyma requires adequate models providing higher accuracy of the results.

The aim of this study was validation of a new mathematical regression model reflecting the relationship between liver weight and body weight for calculation of regeneration coefficient after partial hepatectomy (PHE).

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MATERIALS AND METHODS

Experiments were carried out on 60 male albino rats (175-210 g). Partial hepatectomy was carried out by removal of the central liver lobe under ether narcosis [7]. On the next day after surgery the animals were divided into 3 groups: experimental, control (equal groups), and intact animals. Experimental rats received potassium orotate (100 mg/kg twice a day) orally through a tube for 7 days, because the maximum increment in the liver weight after PHE was observed during this period [6]. Potassium orotate (pyrimidine derivative) is recommended for experimental studies as the agent stimulating regeneration of the liver parenchyma [3]. Controls received the same volume of saline through the tube. Functional activity of the liver was evaluated on day 8 after PHE by the duration of hexenal sleep [11]. Hexenal was injected intraperitoneally (80 mg/kg). After decapitation serum activities of ALT and AST, alkaline phosphatase, total protein, and albumin were measured.

The results were statistically processed using SPSS 11.5 statistical software [2]. The means were

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compared using ANOVA one-way dispersions analysis and Student's *t* test.

RESULTS

Regeneration coefficient was used for quantitative evaluation of the regeneration processes [4]. This coefficient is as follows:

$$K=\frac{P_1-P_2}{P_3},$$

where P_1 is liver weight 7 days after PHE (measured directly), P_2 weight of the liver remaining after hepatectomy, and P_3 weight of the removed liver (measured directly). This weight is estimated as the difference between body weight and initial weight of the liver: $P_2=P_0-P_3$, where P_0 is the initial weight of the liver. Linear regression equation was used for evaluating P_0 [4]: $P_0=b_1\times M+b_0$, where M is animal body weight. The following coefficients were calculated in that study: $b_1=0.036$, $b_0=2.37$. Hence, the authors proposed the formula: $P_0=0.036\times M+2.37$ for calculating the initial weight of the liver.

Body weight and liver weight in intact rats in our study (Fig. 1) served as the basis for regression analysis. If liver weights differed in rats with the same body weight, the mean liver weight was taken. Analysis of dispersions of linear regression equation proposed previously [4] (Table 1) proved reliability of linear relationship ($p=0.019$). On the other hand, b_0 coefficient does not differ from zero ($p=0.132$), and hence, linear equation is inadequate for approximation of experimental data. It is hardly possible that liver weight ($P_0=b_0$) remains 1.96 g in a rat with body weight equal to zero ($M=0$). Therefore we carried out regression analysis with linear equation without constant ($P_0=b_1\times M$; Table 1). The level of significance sharply increased for the difference from zero b_1 coefficient. The level of significance of regression relationship therefore also increased. Hence, the $P_0=0.033\times M$ is the optimal model reflecting the relationship between liver weight and body weight. This model was used in our further estimations. On day 7 after PHE the liver regeneration coefficient in animals treated with potassium orotate was higher than in controls (Table 2). Liver hypertrophy was paralleled by recovery of its functional activity. The duration of hexenal-induced sleep (reflecting the state of the microsomal system) decreased by 51% in rats treated with potassium orotate after PHE in comparison with controls. Serum level of alkaline phosphatase returned tu normal and a trend to normalization of albumin concentration was observed. Blood levels of transaminases and total protein in experimental and control animals virtually did not differ from those in intact animals. These results are in line with published data on potassium orotate capacity to stimulate natural regeneration of hepatocytes and restore their functional activity [3].

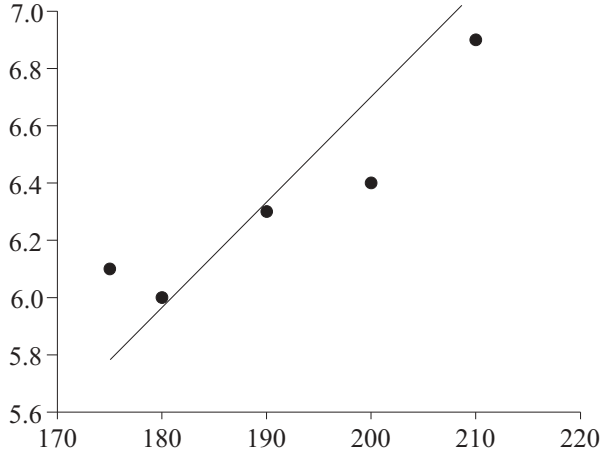

Fig. 1. Relationship between rat liver weight and body weight expressed by an equation $P_0=0.033\times M$. The line is estimated straight line, dots are experimental values. Abscissa: rat body weight (M); ordinate: liver weight (P_0).

Hence, estimation model $P_0=0.033\times M$ reflecting the relationship between liver and body weights can be recommended for estimation of regeneration coefficient after PHE in screening of potential hepatoprotectors for quantitative evaluation of regeneration processes. Presumably, the values of b_1 coefficient can vary. We therefore recommend to select

TABLE 1. Regression Analysis of Relationship between Rat Liver and Body Weights

Linear relationship	Coefficients	Coefficient value	For value other than zero (p)	For analysis of dispersions in the presence of regression (p)
$P_0=b_1\times M+b_0$	b_1	0.023	0.019	0.019
	b_0	1.961	0.132	
$P_0=b_1\times M$	b_1	0.033	0.00001	0.00001

TABLE 2. Effects of Potassium Orotate after PHE ($M \pm m$)

Parameter	Intact animals	PHE+saline	PHE+potassium orotate
Regeneration coefficient		1.43±0.05	1.580±0.042^{+}
Duration of hexenal sleep, min	21.4±1.6	56.8±8.8*	28.8±1.9^{+}
Alkaline phosphatase, U/liter	428.0±20.6	838.6±148.9*	525.6±21.9^{+}
ALT, U/liter	78.02±1.14	71.82±6.11	75.22±2.47
AST, U/liter	254.25±1.89	242.00±6.14	220.50±14.7
Total protein, g/liter	61.25±0.75	63.68±2.32	65.75±1.70
Albumin, g/liter	26.28±0.29	18.67±1.36*	22.73±0.42

Note. $p<0.05$ compared to *intact animals, $^{+}$PHE+saline.

a random representative sampling (at least $n=10$) from the entire group of rats used in the experiment and carry out appropriate regression analysis by the formula $Y=b_1 \times X$ for more accurate evaluation of b_1 coefficient.

REFERENCES

1. O. Yu. Abakumova, N. G. Kutsenko, and L. M. Fedorova, *Vestn. Rossiisk. Akad. Med. Nauk*, No. 5, 36-41 (1996).
2. A. Bryul' and P. Tsefel', *SPSS Packet: Last Word for Analysis, Processing, and Presentation of Statistical Data in Marketing, Sociology, and Medicine* [in Russian], Ed. by V. E. Molot, Moscow, St. Petersburg, Kiev (2002).
3. A. I. Vengerovskii, I. V. Markova, and A. S. Saratikov, *Manual on Experimental Preclinical Studies of New Drugs* [in Russian], Ed. by V. P. Fisenko (2000), pp. 228-231.
4. V. V. Gaivoronskaya, S. V. Okovityi, E. B. Shustov, and A. V. Smirnov, *Eksp. Klin. Farmakol.*, **63**, No. 5, 34-36 (2000).
5. S. D. Podymova, *Liver Diseases. Manual for Physicians* [in Russian], Moscow (1999).
6. B. P. Salopaev, *Regeneration, Adaptation, Homeostasis* [in Russian], Gorky (1990).
7. G. M. Higgins and R. M. Anderson, *Arch. Pathol.*, **12**, 188-202 (1931).
8. G. K. Michalopoulos and M. C. De Frances, *Science*, **267**, 60-66 (1997).
9. M. Rizetto, *Ital. J. Gastroenterol. Hepatol.*, **31**, 781-793 (1999).
10. L. Schiaffonati and L. Tiberio, *Liver*, **17**, No. 4, 183-191 (1997).
11. W. C. Verly, *The Control of Liver Growth*, New York (1976).