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ERK 1/2 activation does not contribute to increased survival mediated by bone marrow cells after 90% partial hepatectomy in Wistar rats

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ABSTRACT

We investigated the influence of bone marrow cells upon activation of ERK ½ in an animal model of 90% PH. Phosphorylated ERK 1/2 was evaluated by western blot. No differences were found between the groups. Thus, increased survival does not appear to be mediated by ERK 1/2 activation.

Keywords: *MAP Kinase Signaling System; stem cells; acute liver failure*

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Acute liver failure (ALF) is defined as a sudden loss of hepatic function leading to jaundice, coagulopathy and encephalopathy in previously healthy individuals. ALF can originate from different causes, such as toxic or viral agents and resection of liver mass[1].

Cell therapy protocols with bone marrow-derived cells have shown promising results increasing the speed of hepatic regeneration[2-4]. The mechanism of action of these cells is not completely understood but different mechanisms have been suggested, including paracrine effects[3,5,6]. In a previous study from our group we have shown that encapsulated whole-bone marrow cells (WBM) increase 10-day survival after 90%HP in 50% when compared with the control group (5%) by decreasing the expression of liver regenerating factors[6]. A previous observation that decreased liver regeneration rate is beneficial after massive hepatocyte loss has been reported by Ninomyia et al.[7]. These authors suggest that inhibition of ERK1/2 leads to a balanced cell division between hepatocytes and sinusoids epithelial cells. In order to investigate if this mechanism could be operating in our model we analyzed ERK 1/2 and AKT phosphorylation status and mitotic index in 90%HP animals receiving encapsulated WBM.

METHOD

To achieve our goals, we used samples of liver obtained from the study published by Uribe-Cruz et al.[6]. Briefly, *Wistar* rats were submitted to 90%PH. WBM cells were isolated from donor rat's and were encapsulated as described by Baldo et al.[8] and Lagranha et al.[9] respectively without prior culture. Microcapsules were implanted in the peritoneum immediately after 90% partial hepatectomy. WBM group received $3x10^7$ cells encapsulated in semipermeable alginate beads, whereas control group received empty capsules (EC). Animals were euthanized at 6, 12, 24, 48, and 72 hours post-surgery (n=5 animals/time/group). Part of the remaining liver was snap frozen in liquid nitrogen then stored at -80ºC. This study was approved by the Ethics Committee on Animal Research at Hospital de Clíncas de Porto Alegre (GPPG-12-0114).

ERK 1/2 activation was analyzed in liver extracts (0.2 g) macerated in 1 mL lysis buffer (HEPES 0,25 mM, Tris 10 mM, EDTA 1 mM, Na3VO 1mM e NaF 10 mM em ph 7,4) containing protease inhibitors (1%) and centrifuged

for 30 minutes at 15000 g at 4°C. Protein quantity was determined by Bradford assay[10]. After 5 minutes at 100 °C samples were separated on PAGE 10% and transferred to PVDF membrane (MiliPore) by wet blotting. Membranes were blocked in solution containing 5% powdered milk diluted in PBS-Tween (0.05%) for 30 minutes and then hybridized with antibodies for phospho-ERK 1/2 (Cell Signaling Technologies, USA) at 4 ºC for 12 hours. Membranes were exposed to horse-raddish peroxidase conjugated secondary antibody (Sigma-Aldrich, USA) for 1 hour at room temperature. Membranes were revealed with ECL kit (BioRad). The density of the specific bands was quantified with imaging density software (Image J, USA).

Statistical analysis was performed on PASW Statistic 18. Results are expressed as mean with SD and analyzed using One Way ANOVA, P values p<0.05 were considered significant.

RESULTS

The expression pattern of phosphorylated ERK 1/2 at different time points is shown in Figure 1. An increase in activated (phosphorylated) ERK 1/2 at 12 hours was observed for EC group compared to 6, 24 and 72 hours after 90% PH (P<0.05); whereas WBM group showed an increase at 12 hours when compared at 6 and 72 hours only (p<0.05). No difference was found on ERK 1/2 at any time point between both groups, although there was a trend for increase in phosphorylated ERK 1/2 at 24 hours after 90% PH in WBM group (p=0.07).

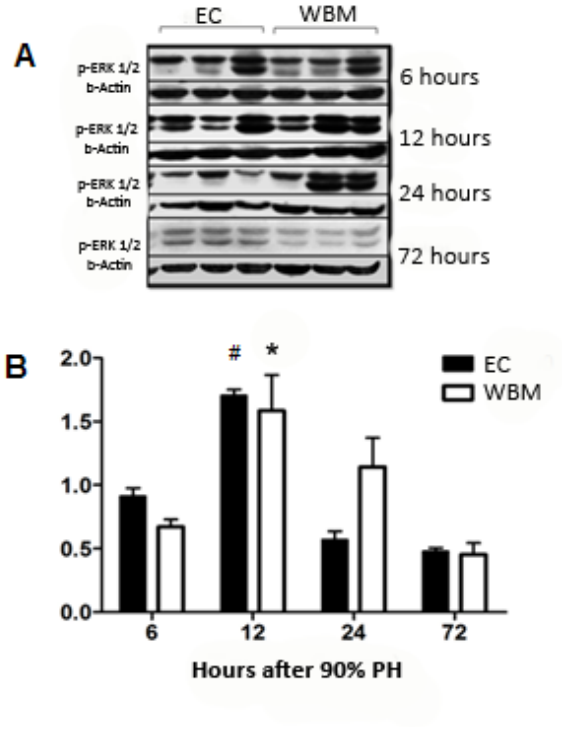

Figure 1: (A) Western Blot showing ERK 1/2 activation in WBM and EC groups at 6, 12, 24 and 72 hours after 90% PH; (B) Relative intensity of ERK 1/2 bands (normalized by Actin bands) of both groups in the same time points. # p<0.05 72 vs 6 and 12 hours EC group and *p<0.05 72 vs 6 and 12 hours WBM group (one-way ANOVA).

DISCUSSION

The liver has an impressive regenerative capacity due to the ability of mature hepatocytes to enter cell cycle upon damaging stimuli. In a previous work we observed that bone marrow cells entrapped in alginate microcapsules increase 10-day survival in a murine model of 90% HP[6]. The present study was conducted to investigate if the beneficial effect of bone marrow cells could be due to ERK 1/2 activity.

Murata et al.[11] showed a progressive decrease in ERK 1/2 2 and 6 hours after 70% PH in animals with thrombocytosis, suggesting that this is a protective effect mediated by increased platelet levels. On the other hand, Ninomiya et al.[7] showed that blocking ERK 1/2 activation with a specific drug results in decreased hepatocyte proliferation and increased survival. In the present study there was no difference between groups regarding ERK1/2 activation, although there was a trend for increase in phosphorylated ERK 1/2 at 24 hours after 90% PH in the group receiving encapsulated bone marrow cells.

Probably the responses elicited by bone marrow cells vary according to the type of lesion, or even that increased proliferation is dependent on cell-cell contact, which is impossible in our model as cells are encapsulated[6].

In conclusion, the present study showed that other mechanisms must be investigated in order to explain this difference in this model of acute liver failure.

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