Effect of Interferon and Retinoic Acid on Liver Metastasis

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Summary

Previous studies demonstrated the efficacy of All-trans Retinoic Acid (ATRA) and Cyclophosphamide (alone or combined) to block the tumoral development (rabdomyosarcoma S4MH) in the liver of syngeneic rats following partial hepatectomy. This study analyzes the effect of Interferon alpha-2b (IF) in the same animal model, alone and combined with ATRA. IF has reduced by 44% de mean number of liver metastasis, but $p>0.05$. When combined with ATRA the reduction reaches 51% ($p<0.05$), with a more intense effect when considering only those metastasis bigger than 1 mm in diameter (80%, $p<0.05$).

Introduction

The capability of the liver to restore its initial volume after the resection of one of its lobes allows surgeons to excise liver metastasis without functional loss. This regeneration is promoted and controlled by several "growth factors" (hepatic growth factor, epidermal growth factor, transforming growth factor, basic fibroblastic growth factor, etc.), and many of them have also proved to promote the growth ("in vitro and "in vivo") of some tumor cells[1]. Thus, the resection of liver metastasis could stimulate the growth of silent tumor cells remaining in the liver[2]. This has appeared as a new and promising field of investigation for experimental surgery laboratories.

In previous studies, using two different experimental models of liver metastasis in rats, we had found that partial liver resection (alone or combined with the excision of the primary tumor) increases the mean

number of macroscopic liver metastasis in a short term[3-4]. This fact led us to start searching for therapies which could block the growth of tumor cells present in the remaining liver. In a series of experiments we demonstrated the antiproliferative effect of a differentiating drug (All-Trans Retinoic Acid, ATRA) in tumor cells[5] and how this therapeutic effect was enhanced with standard chemotherapy (Cyclophosphamide) [6]. In the current study we have tried to analyze the effect of an immunomodulator (Interferon) on liver metastasis proliferation, with a view to use it combined with the two drugs previously tested.

Materials and Methods

Liver metastasis have been induced by inoculation of 250,000 syngeneic rabdomyosarcoma cells (S4MH) into the spleen of syngeneic male rats (WAG). To avoid the "blocking effect" of the primary tumor on the growth of metastasis, the spleen was removed five minutes later. Ten days later, in order to simulate a metastasectomy, the left lateral liver lobe was excised. To assess the effect of the liver resection, the animals were sacrificed on the 21st day, and the liver and lungs were removed and a count of their macroscopic metastasis was carried out in a "blind" mode. Then, both organs were embedded in paraffin to perform a microscopic metastasis count.

Three different treatments have been tested; each group included 20 animals randomly allocated to drug or vehicle treatment. A first group of rats was treated either with ATRA or its solvent (Clinoleic ®); a second one received either IF or saline; while a third group was given either ATRA + IF or Clinoleic + saline.

Interferon alpha-2b (IF) was administered intraperitoneally (650,000 I.U./kg) every second day from the seventh day of the study until the 21st day. Daily doses of ATRA were administered intraperitoneally (5 mg/kg) over the same period. The mean number of metastasis in the different groups were compared with a T test (GraphPad ®).

Results

In the treated animals of the first group we observed a significant reduction in the number of liver metastasis (54%, $p<0.05$). When analyzing these data dividing the metastasis in two groups, according to its size, a different behavioural pattern was found between small (less than one millimeter wide) and large metastasis (over one millimeter wide). In the small ones the reduction remained bellow 40%, while in the large ones it reached 58% ($p<0.05$, figure 1).

IF has reduced the mean number of liver metastasis by 44%, but this difference is not statistically significant ($p>0.05$) due to great individual variations in the animals of this group (figure 2). When we classify the

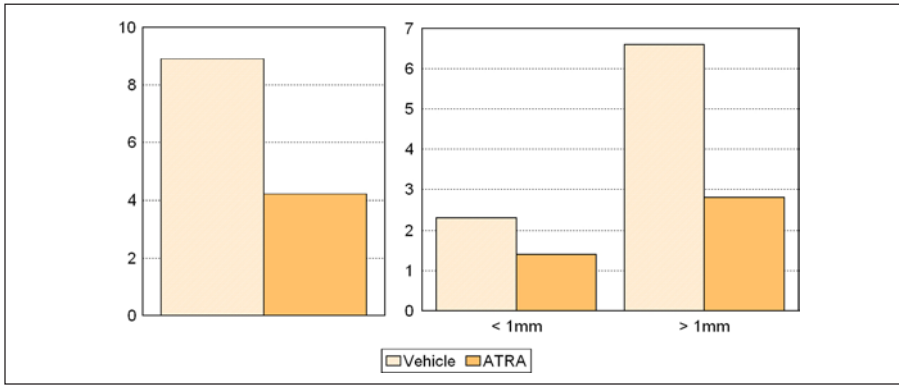

Figure 1. Mean number of macroscopic metastasis in the liver of animals treated with ATRA or vehicle (Clinoleic). On the left graph are represented the total number, while on the right the metastasis are sorted by their size.

metastasis according to their size, we see an effect just the contrary of what was observed in the first group: the greater decrease happens in the small metastasis (with a 57% reduction in their number), compared to a 28% reduction in the large ones ($p>0.05$).

When ATRA and IF were combined the effect was similar to that observed in the first group, with a global reduction in the mean number of liver metastasis up to 51% (figure 3). However, no effect in the number of small metastasis was recorded, while a more intense reduction in the mean number of the large ones was noted (80%, $p<0.05$).

If instead of comparing each treatment against its own control, we put the three of them together (figure 4), we may observe that either of them cuts by half the mean number of liver metastasis, with no signifi-

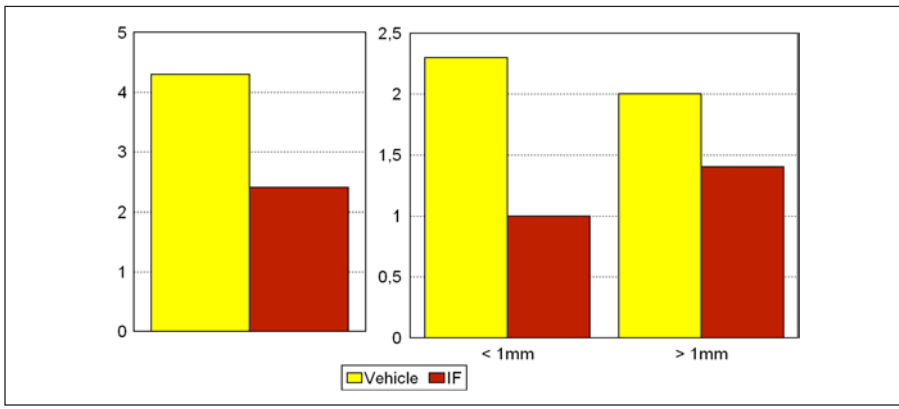

Figure 2. Mean number of macroscopic metastasis in the liver of animals treated with IF or vehicle (saline). On the left graph are represented the total number, while on the right the metastasis are sorted by their size.

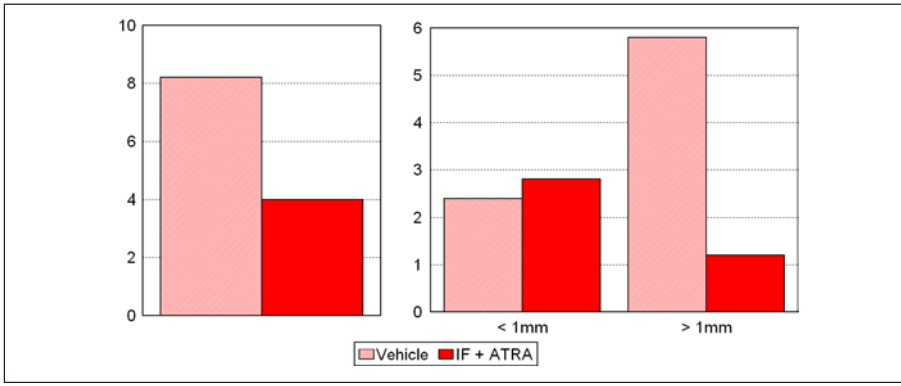

Figure 3. Mean number of macroscopic metastasis in the liver of animals treated with ATRA and IF or vehicle (Clinoleic). On the left graph are represented the total number, while on the right the metastasis are sorted by their size.

cant differences among them ($p>0.05$). The difference between the treatments arise when analyzing their effect in the two types of metastasis, according to their size. Focusing on the big ones, it seems as if there is a summative effect when combining the two drugs. On the other hand, the increased observed in the number of minor metastasis (negative reduction) could be the consequence of a therapeutic effect that strongly inhibits the growth of the small metastasis, without blocking at a similar degree the growth of the microscopic foci to the macroscopic level.

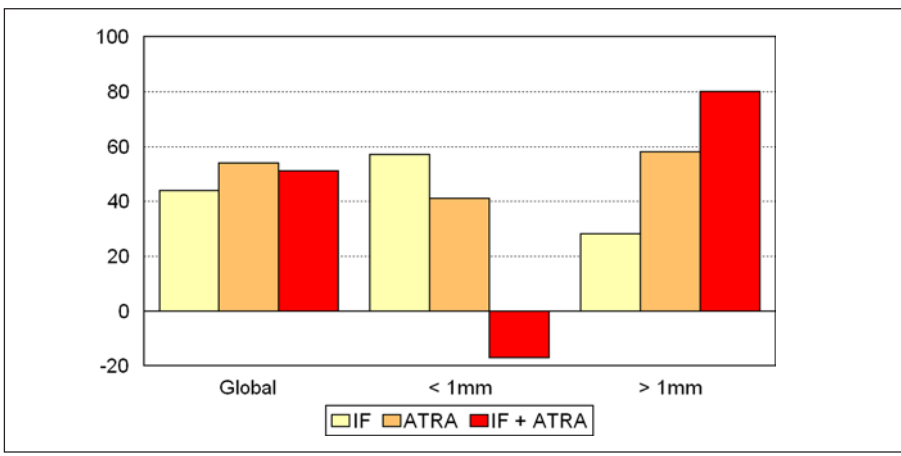

Figure 4. Reduction of the mean number of liver metastasis induced by the different treatments, expressed as percentage with respect to their own controls.

Conclusions

IF has reduced the number of liver metastasis in our experiment,

though our results are not statistically significant. When combining ATRA and IF, a vigorous reduction in the number of large metastasis is achieved.

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