SRF Vacation Scholarship Report – Sophie Nash 2014

How does Chemotherapy Treatment affect Fertility in Pre-pubertal Males?

Introduction:

With the long-term survival rate of cancer patients constantly rising, it is becoming increasingly important to protect the quality of life of the survivors. One aspect of concern is the known detrimental effects that chemotherapy drugs can have on fertility, especially for younger people. This is particularly important for pre-pubertal boys who are not yet producing mature spermatozoa because, at present, there are no established fertility preservation treatments. The aim of this project was to examine the effects of a commonly used chemotherapy drug, irinotecen (through use of its metabolite SN38) on tissue samples of mouse testes in culture, to identify the precise effects of the drug on germ cell number and cell death. This work was done in conjunction with a wider, ongoing study examining several other drug types including cisplatin and doxorubicin. Unpublished results (F Lopes & N Spears, personal communication) have shown that these drugs specifically damage the germ cell population of the testis and the hypothesis being tested here is that irinotecen would do the same. The hope is that by understanding how the different chemotherapy drugs cause damage to the testes, protective treatments can then be developed.

Method:

Neonatal mouse testes (5 days post-birth) were dissected out and cut up into small pieces before being placed in culture for four days and then fixed. Varying concentrations of SN38 were added to the media on day 2 of the culture to some of the samples while those not exposed acted as controls. This method was used to mimic the short exposure to chemotherapy drugs experienced by patients. After fixing and processing, the tissue was histologically sectioned and placed onto slides and underwent immunofluorescent staining. Germ cells were marked using VASA antibodies (Fig 1, red) and cell death was marked using Cleaved Caspase 3 (CC3; Fig 1, green) antibodies and identified using a fluorescent microscope. The resulting images were analysed using the Image J software. For four of the groups, the sample size was 4 and one way ANOVAs were performed. Unpaired, 2-tailed t-tests were then carried out on the groups where significant differences in variance occurred between the treatment groups and the control. The 50ng & 100ng groups only had a sample size of 2 and therefore these results were not included in the statistical analysis.

Results:

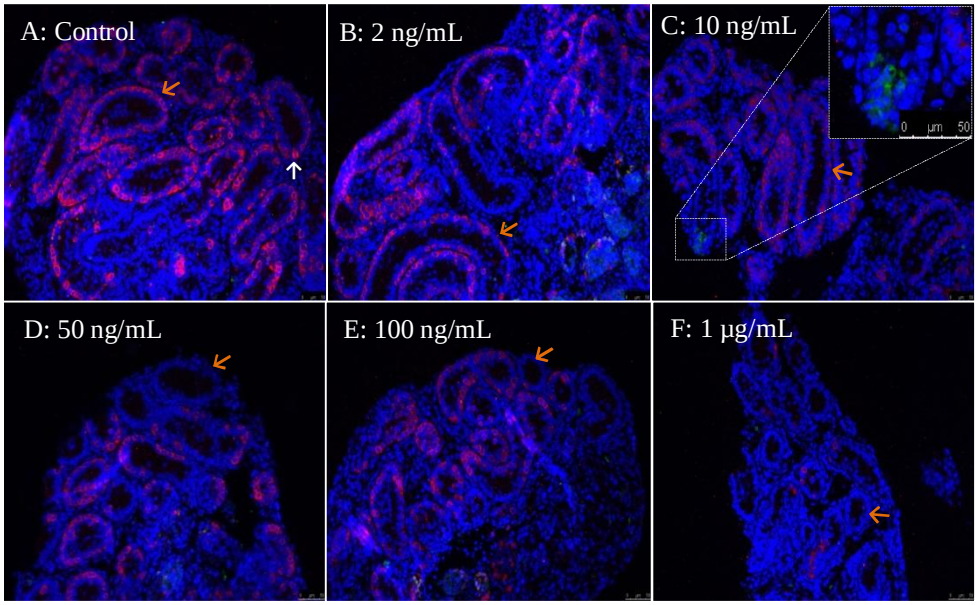
From each sample of tissue, three sections from the beginning, middle and end of the block were analysed after undergoing immunofluorescent histochemistry. The results from each treatment group were then averaged and compared to the controls (see Fig 2).

Analysis of the results showed that the initial presence of SN38 causes a slight decrease in the structural formation of tubules but this decrease wasn't significant and wasn't dose dependent as there were no significant changes between the affected samples. The amount of VASA-positive cells i.e. germ cells decreased significantly ($P<0.0001$) between the control and the highest dose of SN38 (see Fig 1). Interestingly, the initial trend was an increase in germ cells compared to the control up to concentrations of 10ng/mL, but the presence of VASA decreases significantly by 1 μg/mL. The proportion of VASA was calculated both as a percentage of the section area and of the tubule area with a similar trend apparent in each, although the initial increases in VASA Area/Tubule were significant ($P<0.05$) despite being insignificant in the VASA Area/Section results.

Previous unpublished research has shown that germ cells are present in uncultured testes tissue so we can negate the idea that the culture is promoting germ cell proliferation and state that the reduction in VASA is due to the presence of SN38. This decrease in the presence of germ cells could be due to an increase in programmed cell death but there was no corresponding increase in CC3 positive cells. Even though the CC3 marker does not show any increases in apoptosis, other types of cell death that are independent of the CC3 pathway e.g. necrosis may be responsible. It would be advisable to test other apoptotic markers and to also perform a time course analysis of CC3 to check that our observed results were not recorded after expression occurred. If further studies can rule out cell death as the cause of germ cell depletion then another possible

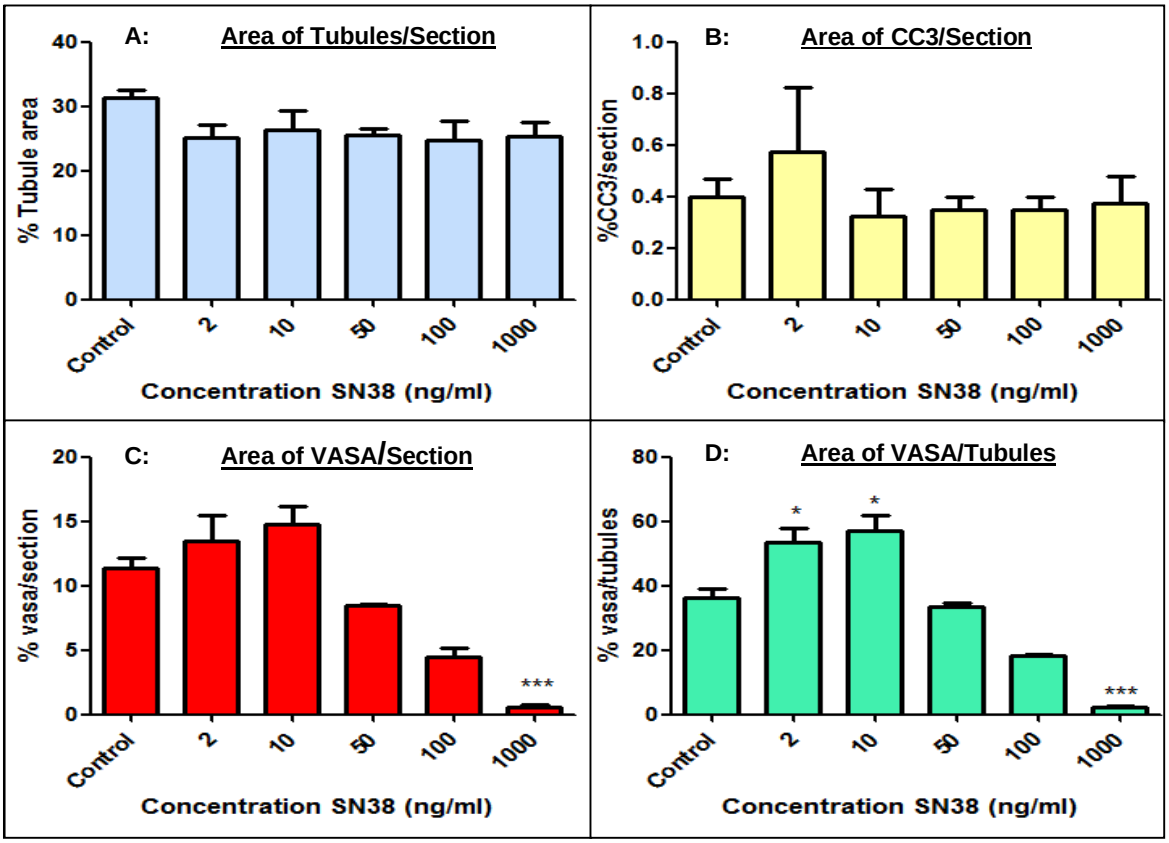
cause would be reduced cell proliferation, which could be identified performing an immunohistochemistry with VASA and BrdU to see if the germ cells have stopped dividing.

Figure 1: Immunofluorescent histochemical imaging of Testis with varying SN38 concentrations



Each treatment group (A-F), including the control, was stained with DAPI (blue), VASA (red) and CC3 (green). The formation of seminiferous tubules is clearly illustrated in each section (orange arrows). Tubule cells stained with VASA i.e. germ cells (A: white arrow) are very evident in low dose groups (A – C) but this appearance depletes with higher concentrations (F). The amount of CC3 between the treatments does not appear to change greatly, with low quantities in each group (C: Insert).

Figure 2: Average values from samples at varying concentrations of SN38



These graphs show the average values from each treatment group with regards to Tubule Area (A), CC3 Area/Section (B), Area VASA/Section (C) and Area VASA/ Tubule Area (D). One-way ANOVA's were performed with no significant differences in variance in either A or B. In both C and D, significant variance differences were found with t-tests determining a significant decrease between the control and the highest SN38 concentration (*** = P<0.001). In D, there was also a significant increase in VASA area between the control and 2ng/mL as well as 10 ng/mL (* = P<0.05). (Error bars = standard error of the mean)

Conclusions:

The results from this project indicate that high concentrations of irinotecan have detrimental effects on germ cell numbers within the testes. The exact nature of how this drug affects germ cells needs further investigation.