4 RESULTS

4.1 VALIDATION STUDY

4.1.1 GENERAL

A total of 84 animals were operated on to provide 70 surviving rats needed for the study. Of these, 68 completed the study. In the 12 week group one animal died of sepsis secondary to an intra-abdominal abscess 4 weeks after surgery, and in the 24 week group another died of sepsis secondary to aspiration 8 weeks after surgery. The timing and cause of death of all rats that were operated on but did not survive until the end of the study are shown in Figure 13.

The body weight of the operated animals fell significantly in the first 4 weeks after surgery, but was regained by week 8. Thereafter, the operated animals weight plateaud for the duration of the experiment as shown in Figure 14. Weight gain was significantly lower in the operated rats compared to the non-operated group throughout the 28-week study.

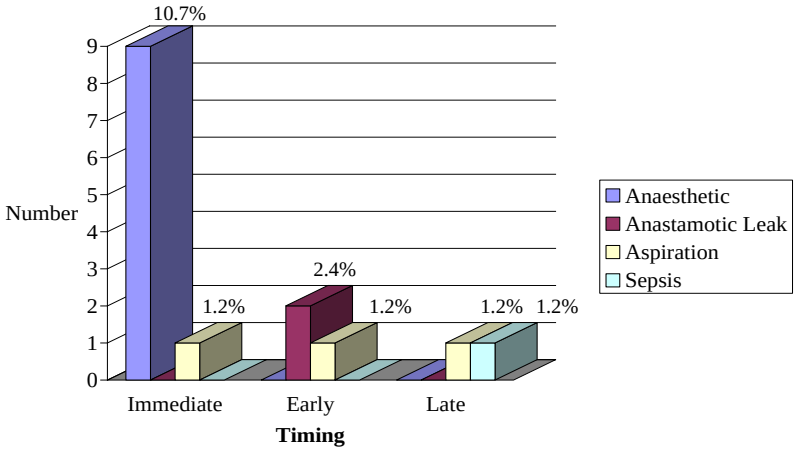


Figure 13. Cause of death of all rats that did not survive until the end of the study. The majority of deaths occurred peri-operatively and were related to anaesthetic complications. Two rats died following randomisation, one from aspiration due to an ischaemic stricture at the site of the anastomosis, and the other from sepsis secondary to an intra-abdominal abscess.

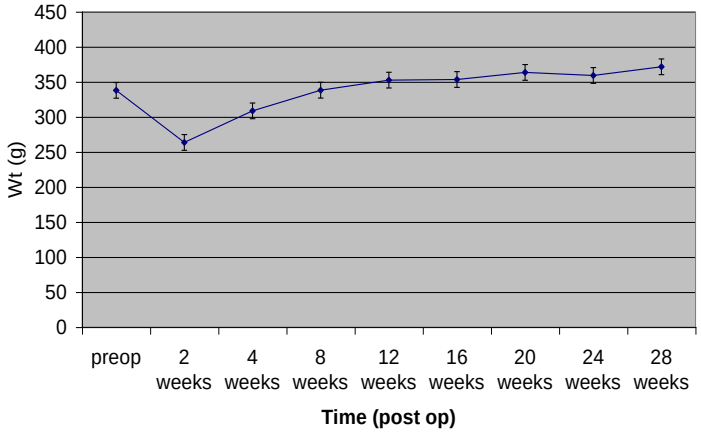


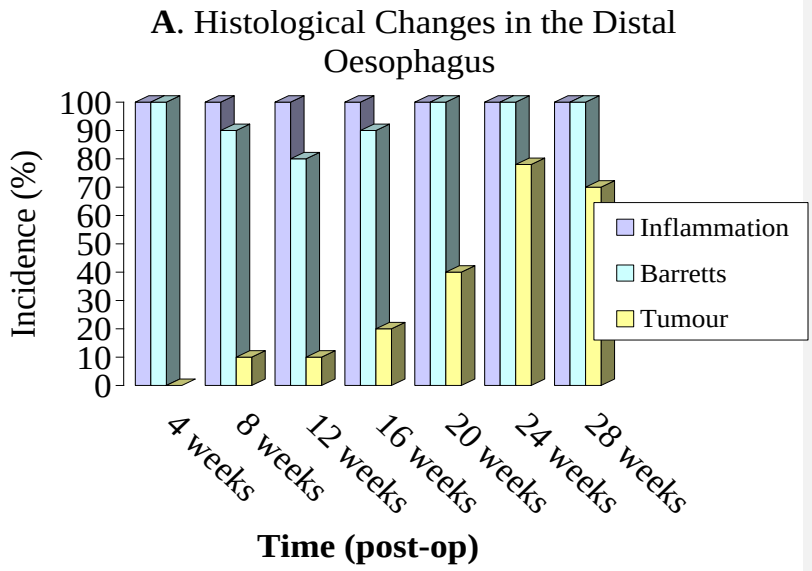
Figure 14. Weight gain of rats following oesophagojejunostomy. Pre-operatively the mean weight of the rats was 342g. In the first two weeks following surgery the mean weight of the rats dropped to 257g. Animals regained their pre-operative weight by 8 weeks after surgery after which their weight plateaud.

4.1.2 HISTOLOGY

All operated animals in each group had severe inflammation of the distal oesophagus with extensive mixed cellular infiltration of the mucosa and submucosa and microscopic ulceration. The proximal extent of oesophageal inflammation marginally increased with time from 3.6 mm at 4 weeks to 4.8 mm by 28 weeks. Severe ulceration of the distal oesophagus proximal to the anastomosis was evident in 90% of rats at 4 weeks. The incidence of severe ulceration fell to 10% by 12 weeks with areas of ulceration being replaced by a regenerative squamous epithelial layer. The regenerative epithelium was hyperplastic (more than double thickness) with acanthosis, had abnormal extensions of the papillae towards the mucosal surface, and a marked parakeratosis. A second peak of severe ulceration was seen at 24 weeks. The late peak in severe ulceration was secondary to underlying carcinoma in all cases.

Barrett's oesophagus was present in all operated animals from 4 weeks. The Barrett's segment was invariably located immediately proximal to the anastomosis. An area of ~~intervening bridge of~~ oesophageal squamous epithelium between the Barrett's segment and jejunal mucosa was observed in most cases. The length of the Barrett's segment increased with time from surgery from 1mm at 4 weeks to 14mm by 28 weeks. The first oesophageal cancer was seen 8 weeks following surgery. The incidence of carcinoma increased with time and 70% had carcinoma by 28 weeks. All carcinomas originated from the oesophagus above the oesophagojejunal anastomosis and were well-differentiated mucinous adenocarcinomas. 84% of carcinomas originated

within an island of Barrett's oesophagus. The macroscopic and microscopic changes in the rat oesophagus following surgery are summarised in Figure 15a and 15b, and demonstrated in Figures 16 and 17.



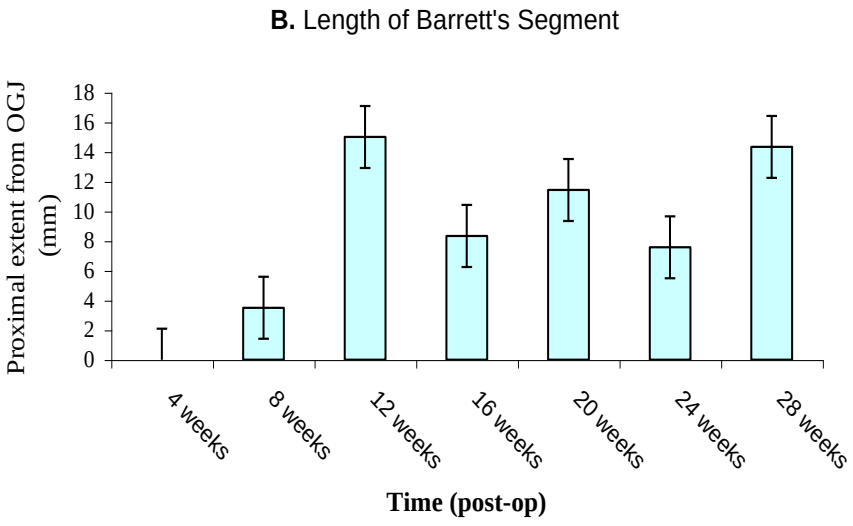
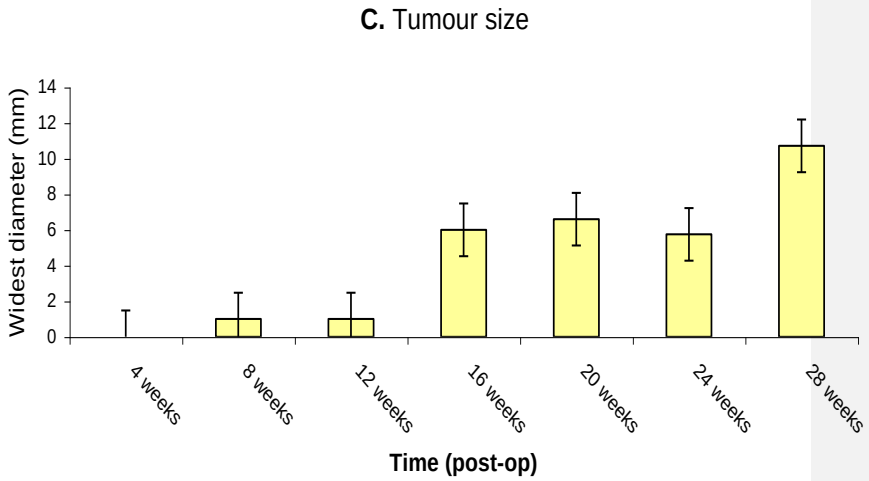


Figure 15a. Morphological changes in the distal oesophagus of rats following oesophagojejunostomy. **(A)** Severe inflammation of the distal oesophagus was present in all rats from 4 weeks. (**A,B**) All rats had Barrett's oesophagus by 4 weeks. The length of Barrett's segment increased from 1mm at 4 weeks to 14 mm by 28 weeks. (**A**) The first adenocarcinoma was seen 8 weeks after surgery.



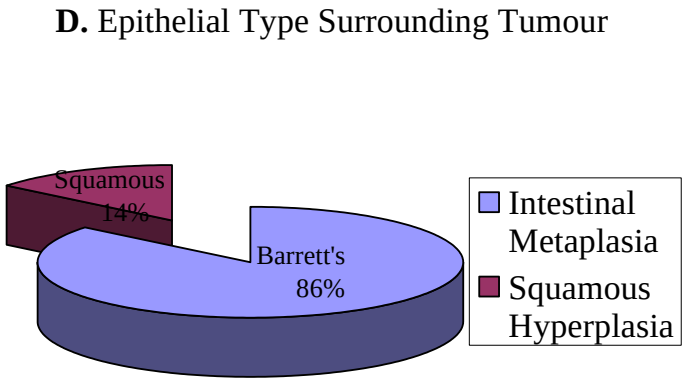


Figure 15b. Morphological changes in the distal oesophagus of rats following oesophagojejuostomy. (**C**) Tumour size (estimated by widest diameter) increased from 1mm at 8 weeks to 11 mm by 28 weeks. (**D**) 86% of adenocarcinoma arose within an island of Barrett's oesophagus.

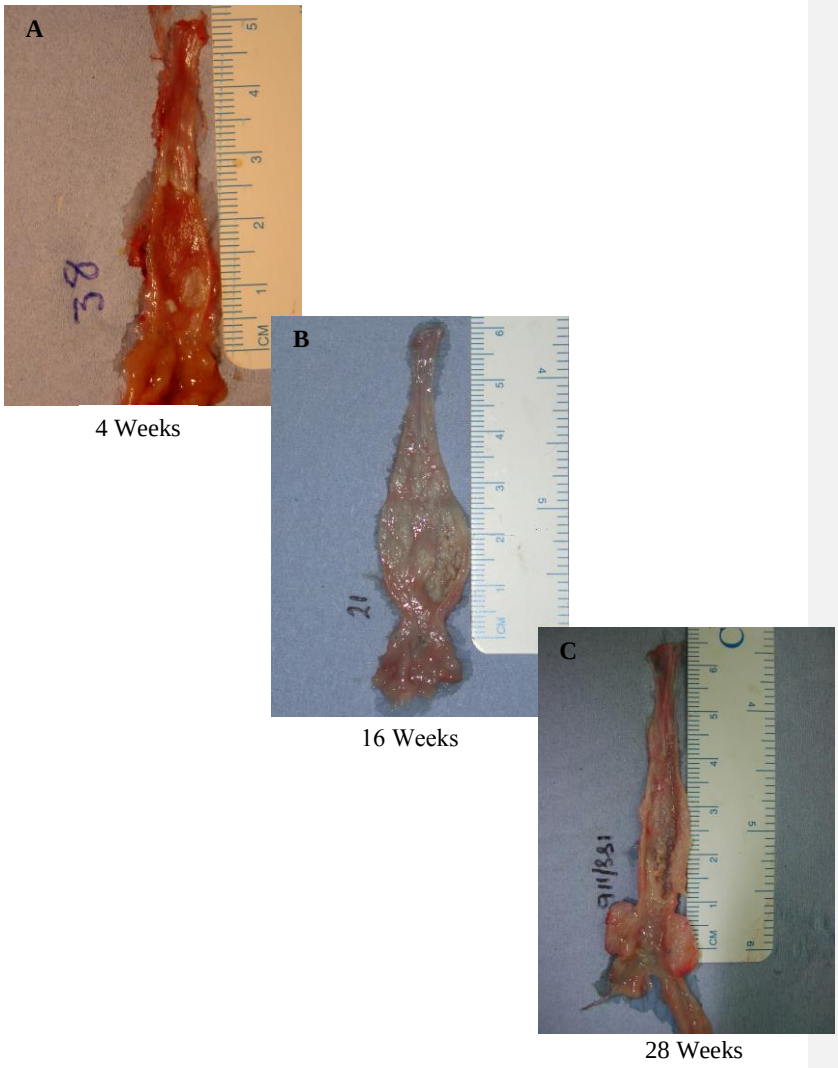


Figure 16. Macroscopic changes in the rat oesophagus following surgery. (**A**) Severe oesophageal inflammation with extensive ulceration at 4 weeks is replaced by thickened white epithelium (proximal oesophagus) and velvety salmon pink epithelium (adjacent to the oesophagojejunal junction) by 16 weeks (**B**). Carcinomas typically develop in the distal oesophagus immediately proximal to the anastomosis (**C**).

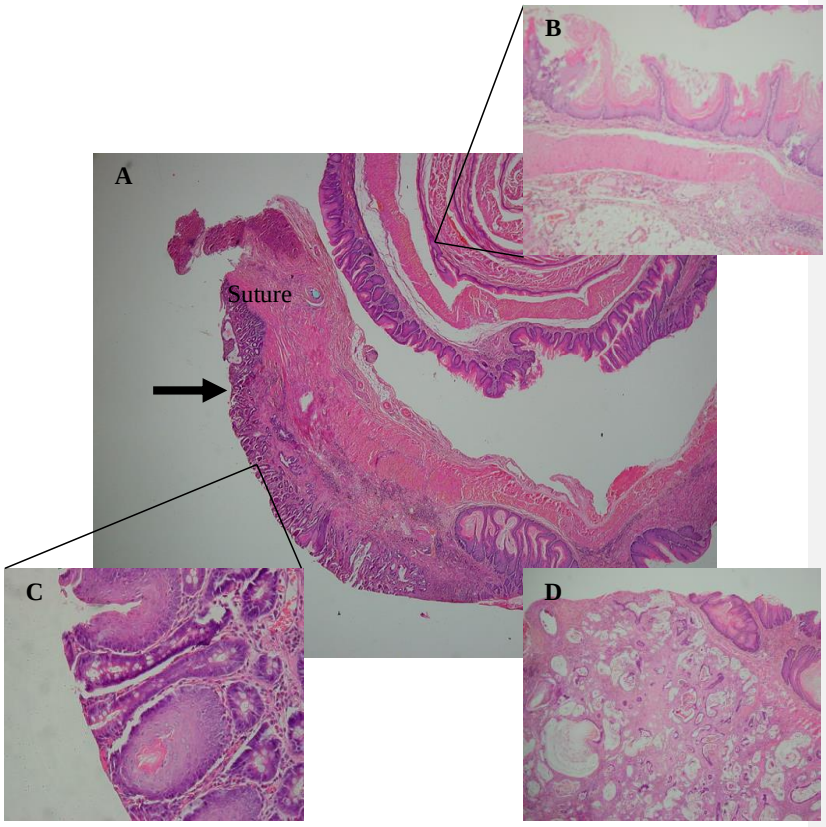


Figure 17. Histological changes in the rat esophagus after oesophagojejunostomy. (**A**) Cross section of oesophagus 12 weeks after surgery (100x magnification). A blue prolene suture used to fashion the anastomosis can be seen. The black arrow indicates the oesophagojejunal junction. (**B**) The proximal oesophageal epithelium is hyperplastic, keratinised, and stratified squamous in type (corresponding to the thickened white mucosa seen macroscopically). (**C**) Barrett's oesophagus (600x magnification). Columnar epithelium with intestinal metaplasia is seen proximal to the anastomosis (Correspoding to the velvety salmon pink mucosa seen macroscopically). Stratified squamous epithelium of the oesophagus is seen both proximally and distally to this segment. (**D**) Typical appearance of well differentiated adenocarcinoma seen in this model (This photomicrograph does not relate to the oesophageal cross section shown in **A**)

4.1.3 CpG ISLAND METHYLATION

Quantitative analysis of CpG island methylation in the *ESR-1*, *p16*, and *HPP1* promoter regions demonstrated that CpG island methylation occurred at all three alleles in all tissue subtypes we examined in this model, but at low levels. The *ESR-1* allele was more heavily methylated than both the *p16* and *HPP1* alleles in all tissue sub-types, and at each time point after surgery.

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4.1.3i *ESR-1*

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There was a significant increase in the level of *ESR1* promoter methylation in both Barrett's oesophagus (2.36% SEM 0.35 p=<0.001) and tumour (3.12% SEM 0.67 p=<0.001) compared to both oesophageal squamous epithelium (0.53% SEM 0.16) and jejunal mucosa (0.64% SEM 0.15) at 28 weeks following surgery. The degree of methylation observed in Barrett's tissue at earlier time points (0.75% SEM 0.14 and 0.66% SEM 0.13 at 4 and 16 weeks following surgery respectively) was similar to the methylation levels observed in both the oesophageal squamous epithelium and jejunal epithelium at the same time points. The results are illustrated in Figure 18.

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4.1.3ii *p16*

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Methylation of the *p16* promoter region was seen in all tissue subtypes and at all time points, but at a very low level. A small but significant increase in the level of methylation was seen in tumour (0.11% SEM 0.02) compared to oesophageal squamous epithelium (0.006% SEM 0.003) 28 weeks after surgery (p=0.0001). A small but non-significant increase in methylation levels was seen in Barrett's oesophagus throughout the time course compared to oesophageal squamous

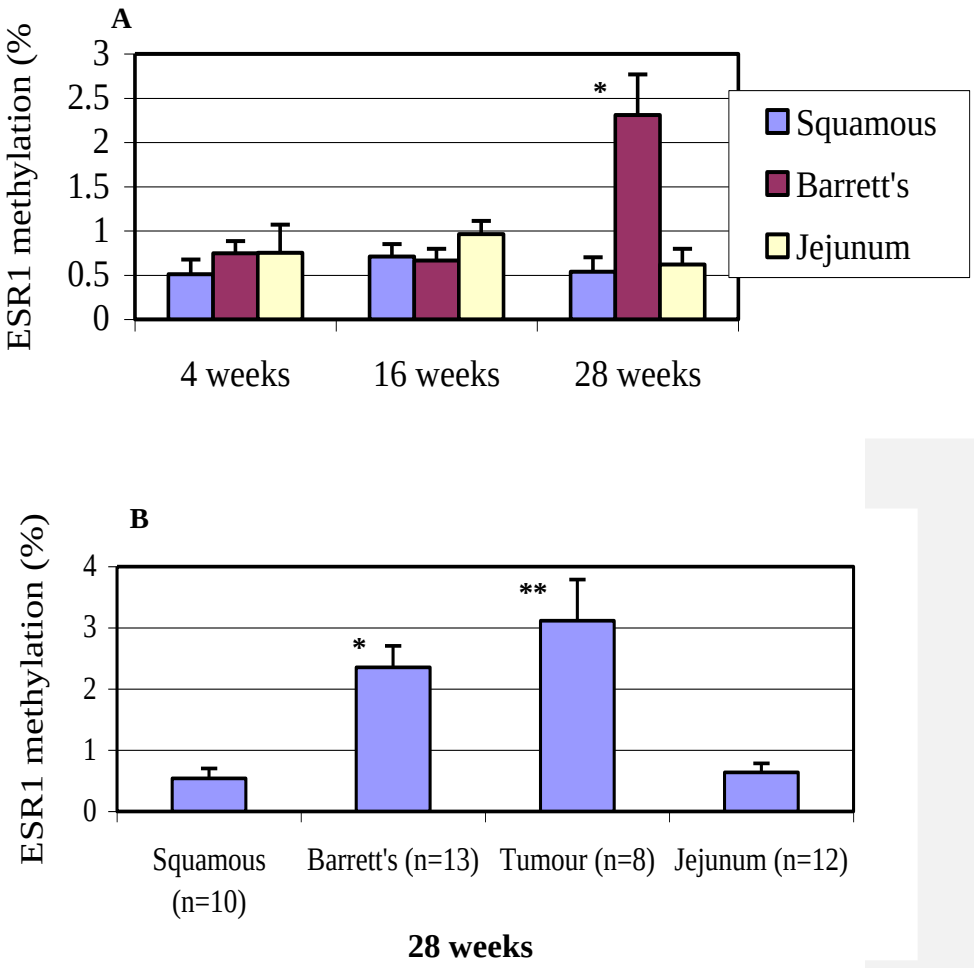

Figure 18. Mean % methylation levels following quantitative analysis of CpG island methylation in the *ESR1* promoter region of the rat oesophagus at 4, 16 and 28 weeks after surgical induction of reflux disease. There was a significant increase in % methylation in both *****Barrett's oesophagus **(A)** and ******EAC **(B)** at 28 weeks compared to both oesophageal squamous and jejunal epithelium ($p<0.001$). The overall degree of CpG island methylation in the *ESR1* promoter in both Barrett's and tumour tissue is low compared to methylation levels reported in human disease

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epithelium. Four weeks after induction of reflux, methylation levels in Barrett's oesophagus were 0.016% (SEM 0.006) compared to 0.009% (SEM 0.004) in oesophageal squamous epithelium (p=0.3). This increased to 0.11% (SEM 0.05) methylation compared to 0.01% (SEM 0.006) by 16 weeks in Barrett's oesophagus and oesophageal squamous epithelium respectively (p=0.08). By 28 weeks methylation levels in Barrett's oesophagus were 1.68% (SEM 1.06) compared to 0.006 (SEM 0.003) in the oesophageal squamous epithelium (p=0.24). These results are shown in figure 19.

4.1.3iii *HPP1*

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The *HPP1* promoter was also methylated in all tissue subtypes and at all time points, but again at very low levels. Methylation levels in Barrett's oesophagus were very low, and equivalent, in all tissue subtypes 4 weeks after induction of reflux, but were significantly greater in Barrett's oesophagus at both 16 weeks and 28 weeks compared to oesophageal squamous epithelium. The levels of methylation in Barrett's oesophagus at 16 weeks was 0.28% (SEM 0.07) compared to methylation levels of 0.04% (SEM 0.02) in oesophageal squamous epithelium (p=0.007). By 28 weeks the methylation levels of Barrett's oesophagus had increased to 0.8% (SEM 0.17) compared to levels of 0.016% (SEM 0.007) in oesophageal squamous epithelium (p=0.0009). The level of methylation in Barrett's oesophagus at 28 weeks was significantly higher than the methylation levels in Barrett's oesophagus at both 16 weeks (p=0.02), and 4 weeks (p=0.0004). Methylation was also significantly increased in tumour (0.59% SEM 0.09 p=0.0001) compared to oesophageal squamous epithelium. The results are shown in Figure 20.

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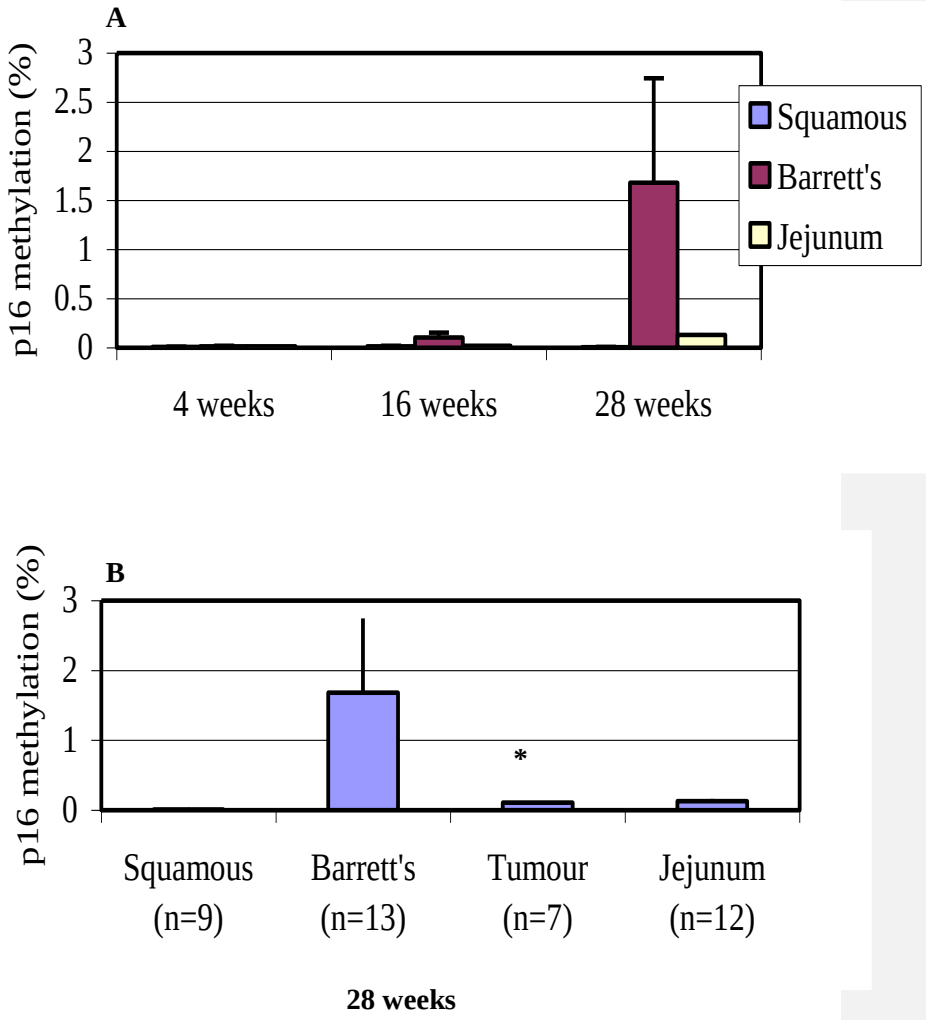

Figure 19. Mean % methylation levels following quantitative analysis of CpG island methylation in the *p16* promoter region of the rat oesophagus at 4, 16 and 28 weeks after surgical induction of reflux disease. (**A**) Increased levels of *p16* methylation were present in Barrett's oesophagus from 4 weeks, and the level of methylation increased progressively to 28 weeks compared to both oesophageal squamous and jejunal epithelium. However, this increase was not statistically significant. (**B**) *p16* methylation occurred at a significantly higher level in tumour compared to oesophageal squamous epithelium at 28 weeks (*p=0.0001).

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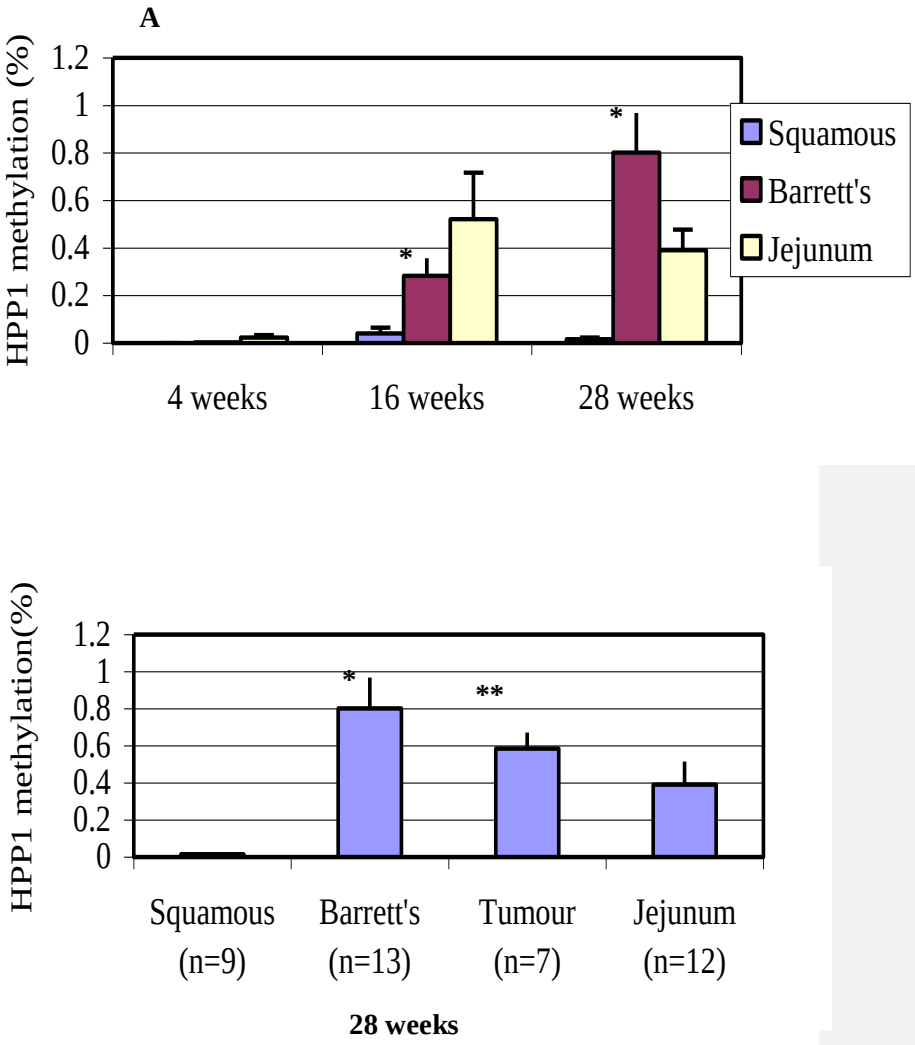

Figure 20. Mean % methylation levels following quantitative analysis of CpG island methylation in the *HPP1* promoter region of the rat oesophagus at 4, 16 and 28 weeks after surgical induction of reflux disease. (**A**) There was a progressive and significant increase in % methylation in Barrett's oesophagus from 16 weeks compared oesophageal squamous epithelium (*****p<0.001). (**B**) Significantly higher levels of methylation were also seen in tumour (******p<0.001). However, the overall levels of methylation of *HPP1* are much lower than we have observed in humans (unpublished results)

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4.1.4 GLOBAL GENE TRANSCRIPTION

Differential gene expression by more than 1.5 fold occurred in 1225 genes at 4 weeks (374 genes up-regulated, 851 genes down-regulated), 1406 genes at 16 weeks (381 genes upregulated, 1025 genes down regulated), and 2098 genes at 28 weeks (611 genes upregulated, 1487 genes down regulated) compared to controls. A total of 2900 genes were differentially expressed in adenocarcinoma compared to controls (903 genes upregulated, 1997 genes down regulated).

Sixteen genes reported to undergo transcriptional dysregulation in human BO were represented on the rat array. At 28 weeks after surgery, changes in relative levels of gene expression occurred in 11 (69%) of these genes in at least one of three experimental animals compared to controls. Nine (82%) of the 11 genes with altered levels of expression in the rat model were over or under expressed in a similar fashion to the expression changes described in human BO. Transcriptional dysregulation of the 11 genes observed to have altered levels of expression in BO at 28 weeks after surgery occurred in a stepwise fashion. Four weeks after surgery 1 gene (*HSP27*) had a different pattern of expression compared to controls. By 16 weeks 4 genes (*HSP27*, *Bcl-2*, *apc*, and *c-myc*) were differentially expressed. The genes and relative changes in expression level with time from surgery are shown in figure 21.

Twenty-three genes reported to undergo transcriptional dysregulation in human OAC were represented on the rat array. Changes in relative levels of gene expression occurred in 17 (74%) of these genes in at least one of three

	Gene	Rat Barrett's			Human Barrett's	Homology
		Time from surgery (weeks)				
		4	16	28		
Irvani et al 2003	*Bcl-x*	→	→	→	↓	
Raouf et al 2003	*Bcl-2*	→	↓	↓	↓	Y
Clement et al 2006	*apc*	→	↑	↑	↓	
Arul et al 2000	*mucin*	→	→	↑	↑	Y
Lord et al 2000	*Telomerase*	→	→	→	↑	
Brabender et al 2001	*c-myb*	→	→	↑	↑	Y
Doak et al 2004	*HSP27*	↓	↓	↓	↓	Y
al-Kasspooles et al 1993	*EGFR*	→	→	→	↑	
Brabender et al 2002	*GSTPI*	→	→	↑	↓	
Tselepis et al 2003	*c-myc*	→	↓	→	↑	
Arber et al 1996	*cyclin D1*	→	→	↑	↑	Y
Swami et al 1995	*E cadherin*	→	→	→	↓	
Irvani et al 2003	*c-src*	→	→	↑	↑	Y
Lord et al 2001	*RAR-alpha*	→	→	↓	↓	Y
Brabender et al 2003	*SPARC*	→	→	↑	↑	Y
Brabender et al 2001	*ornithine decarboxylase*	→	→	↑	↑	Y
	Upregulated	**0**	**1**	**8**	**9**	
	No change	**15**	**12**	**5**	**0**	
	Down regulated	**1**	**3**	**3**	**7**	

Figure 21. Changes observed using cDNA microarrays in the level of expression of genes in Barrett's oesophagus in the rat model compared to human disease. Barrett's tissue from 3 separate rats was analysed at each time point. Transcriptional dysregulation of *HSP27*, *Bcl-2*, *apc*, *myc* and *ras* occurred early in the time course. The change in level of expression of each gene is indicated by an arrow (↑ = increased expression, ↓ = decreased expression, and → = no change in expression compared to controls).

experimental animals compared to controls. Thirteen (76%) of the 17 genes with altered levels of expression in the rat model were over or under expressed in a similar fashion to the expression changes described in human OAC. The genes and relative changes in expression level are shown in figure 22.

	Gene	Rat adenocarcinoma	Human adenocarcinoma	Homology
Irvani et al 2003	*Bcl-x*	↓	↓	Y
Raouf et al 2003	*Bcl-2*	↓	↓	Y
Clement et al 2006	*apc*	↑	↓	
Miller et al 2003	*DYRK2*	→	↑	
Salmela et al 2001	*TIMP3*	↑	↑	Y
Salmela et al 2001	*TIMP1*	↑	↑	Y
Arul et al 2000	*mucin*	↑	↑	Y
Lord et al 2000	*Telomerase*	→	↑	
Brabender et al 2001	*c-myb*	↑	↑	Y
Doak et al 2004	*HSP27*	↓	↑	
al-Kasspooles et al 1993	*EGFR*	→	↑	
Brabender et al 2002	*GSTPI*	↑	↓	
Tselepis et al 2003	*c-myc*	→	↑	
Arber et al 1996	*cyclin D1*	↑	↑	Y
Swami et al 1995	*E cadherin*	→	↓	
Galiana et al 1995	*ras*	↑	↑	Y
Irvani et al 2003	*c-src*	↑	↑	Y
Morgan et al 1999	*mdm2*	↓	↑	
D'Errico et al 2000	*TGFalpha*	→	↑	
Yoshida et al 1993	*TGFbeta receptor*	↓	↓	Y
Lord et al 2001	*RAR-alpha*	↓	↓	Y
Brabender et al 2003	*SPARC*	↑	↑	Y
Brabender et al 2001	*ornithine decarboxylase*	↑	↑	Y
	Upregulated	**11**	**16**	
	No change	**6**	**0**	
	Down regulated	**6**	**7**	

Figure 22. Changes observed using cDNA microarrays in the level of expression of genes implicated in the pathogensis of oesophageal adenocarcinoma in the rat model compared to human disease. The change in level of expression of each gene is indicated by an arrow (↑ = increased expression, ↓ = decreased expression, and → = no change in expression compared to controls).

4.2 INTERVENTION STUDY

4.2.1 GENERAL

4.2.1i *Pre-Initiation Study*

Within the *pre-initiation group* 25 (78%) of the 32 rats survived to complete the study. 12 rats (75%) in the aspirin group completed the study. Two rats died immediately post-operatively due to complications of the anaesthetic, and 2 rats died at 4 and 5 weeks respectively from sepsis secondary to an intra-abdominal abscess. In the quercetin group 13 rats (81%) completed the study. One rat died peri-operatively due to anaesthetic complications, 1 rat died at 5 weeks due to sepsis secondary to an intra-abdominal abscess, and 1 rat died at 16 weeks due to sepsis secondary to aspiration after developing an inflammatory stricture proximal to the oesophagojejunostomy.

4.2.1ii *Long-term Study*

A total of 132 rats were operated on to provide 105 surviving rats needed for the *long-term* study.

Twenty-eight rats (80%) in the aspirin group completed the study. Three rats died at 8, 9, and 22 weeks respectively of sepsis secondary to an intra-abdominal abscess, and 3 rats died at 16, 19, and 20 weeks respectively of sepsis secondary to aspiration. Two of the rats that aspirated had a food bolus impacted in the distal oesophagus with no evidence of a stricture, and one had an inflammatory stricture just proximal to the oesophagojejunostomy with Barrett's oesophagus but no evidence of tumour. Finally, one rat in the aspirin group died at 19 weeks with no obvious cause found at post mortem.

In the quercetin group 28 rats (80%) completed the study. Two rats died at 8 and 9 weeks respectively from sepsis secondary to an intra-abdominal abscess. Four rats died of sepsis secondary to aspiration at 14, 15, 17 and 19 weeks respectively. All four rats had an inflammatory stricture and associated BO in the distal oesophagus with no evidence of tumour. Again, one rat died at 15 weeks with no obvious cause of death found at post mortem.

Thirty rats (86%) completed the study in the control group. One rat died at 5 weeks from sepsis secondary to an intra-abdominal abscess. The remaining four rats died at 5, 8, 17, and 18 weeks of sepsis secondary to aspiration. All 4 rats had an inflammatory stricture with BO proximal to the oesophagojejunostomy, with no evidence of tumour.

The body weight of all operated rats fell significantly in the first 4 weeks after surgery, but was regained by week 8. Thereafter the operated rats gained weight steadily until 28 weeks. Weight gain was similar in all experimental groups throughout the 28-week study as shown in Figure 23.

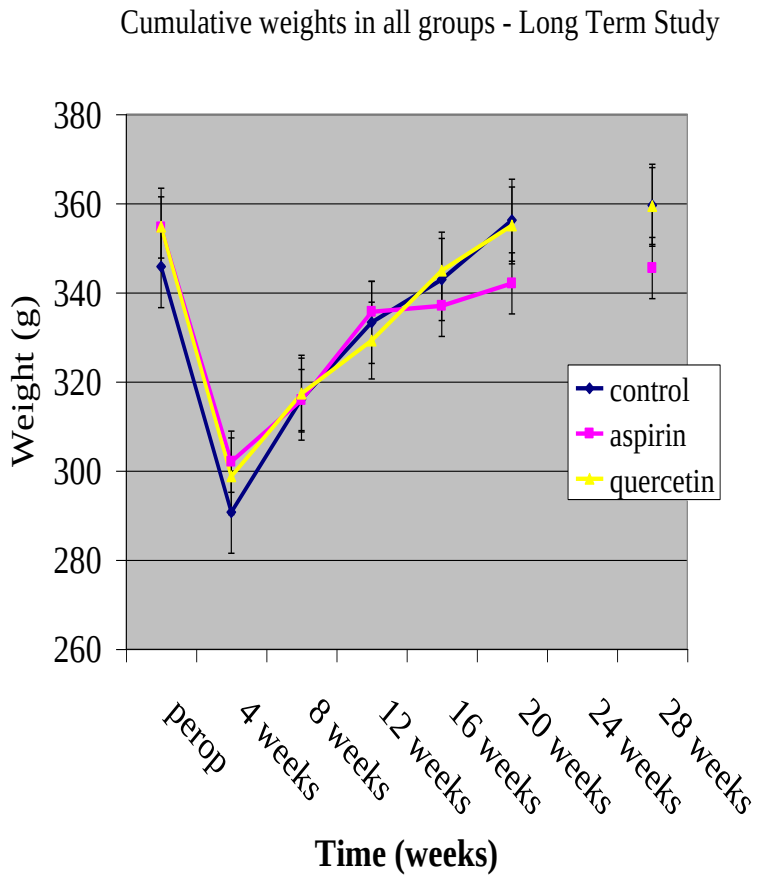

Figure 23. Mean body weight of the rats in each experimental group during the intervention study. There was no significant difference weight gain between the rats in the 3 experimental groups. As observed in the validation study, there was a significant decrease in weight during the first four weeks of the experiment. The animals subsequently regained the lost weight but did not achieve their pre-operative weight until 20 weeks after surgery.

4.2.2 SERUM DRUG CONCENTRATION

Rats receiving 30mg/Kg/24h of aspirin had a mean plasma aspirin concentration of 18.9 mmol/L (range <10-41 mmol/L). A mean plasma quercetin concentration of 0.24mg/L (range 0-2.4mg/L), which includes the sum of both quercetin and its metabolites, was achieved in the rats receiving 70mg/Kg/24h of quercetin. Control animals had undetectable serum and plasma levels of aspirin and quercetin respectively.

4.2.3 OESOPHAGEAL CANCER AND BARRETT'S OESOPHAGUS

4.2.3i General

The histological changes observed in the rat oesophagus during this study were similar to those observed in the validation study. The rats in all experimental groups had severe inflammation of the distal oesophagus with an extensive mixed cellular infiltration of the mucosa and submucosa and microscopic ulceration. Barrett's oesophagus invariably occurred immediately proximal to the anastomosis. An ~~intervening bridg~~area~~e~~ of oesophageal squamous epithelium between the Barrett's segment and jujunal mucosa was observed in most cases. All carcinomas originated from the oesophagus above the oesophagojejunal anastomosis and were well-differentiated mucinous adenocarcinomas.

4.2.3ii Pre-Initiation vs Post-Initiation Intervention

There was no significant difference in the incidence of BO or OAC between rats fed the experimental diet prior to the onset of reflux (*pre-initiation study*) and those commenced on the test diet once reflux was established (*long-term study*) in either the aspirin or quercetin groups. Three of the 12-surviving rats (25%) treated with aspirin in the *pre-initiation study* developed OAC compared to 10 of the 28 (36%) surviving rats in the *long-term study* (p=0.62). All surviving rats in the aspirin group in both the *pre-initiation* and *long-term study* had BO. Within the quercetin group 6 of the 13-surviving rats (46%) in the *pre-initiation study* developed OAC compared to 14 of the 28-surviving rats (50%) in the *long-term study* (p=0.81). All 13 rats (100%) in the *pre-intervention study* had BO compared to 23 of the 28-surviving rats (82%) in the *long-term study*. These results are shown in Figure 24.

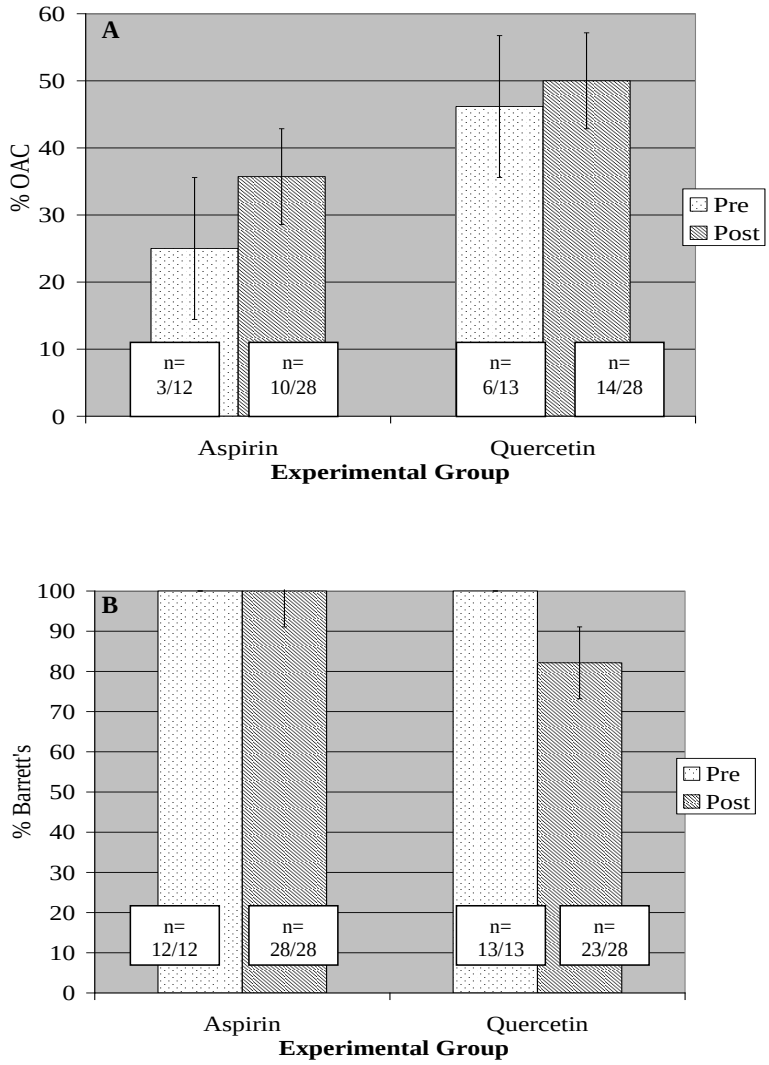

Figure 24. Incidence of OAC and Barrett's oesophagus in rats treated with aspirin or quercetin prior to the onset of reflux (pre-initiation group) compared to rats where treatment did not start until reflux disease was firmly established (long-term group). Timing of treatment with respect to the onset of reflux disease had no significant effect on the incidence of OAC (**A**) or Barrett's oesophagus (**B**) in either the aspirin or quercetin groups.

4.2.3iii Cancer and Barrett's Incidence

The incidence of OAC was significantly lower in aspirin treated rats than in the control group. Thirteen of 40 rats (33%) in the aspirin group developed OAC compared to 18 of 30 (60%) rats in the control group ($p=0.003$). A reduction in the incidence of OAC in quercetin treated rats was also observed with 20 of 41 (49%) rats developing OAC compared to the control group. However, this reduction was not statistically significant ($p=0.24$). There was no significant difference in the incidence of BO in either the aspirin or quercetin treated groups compared to the control group. These results are shown in Figure 25.

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18/30

n=
13/40

n=
20/41

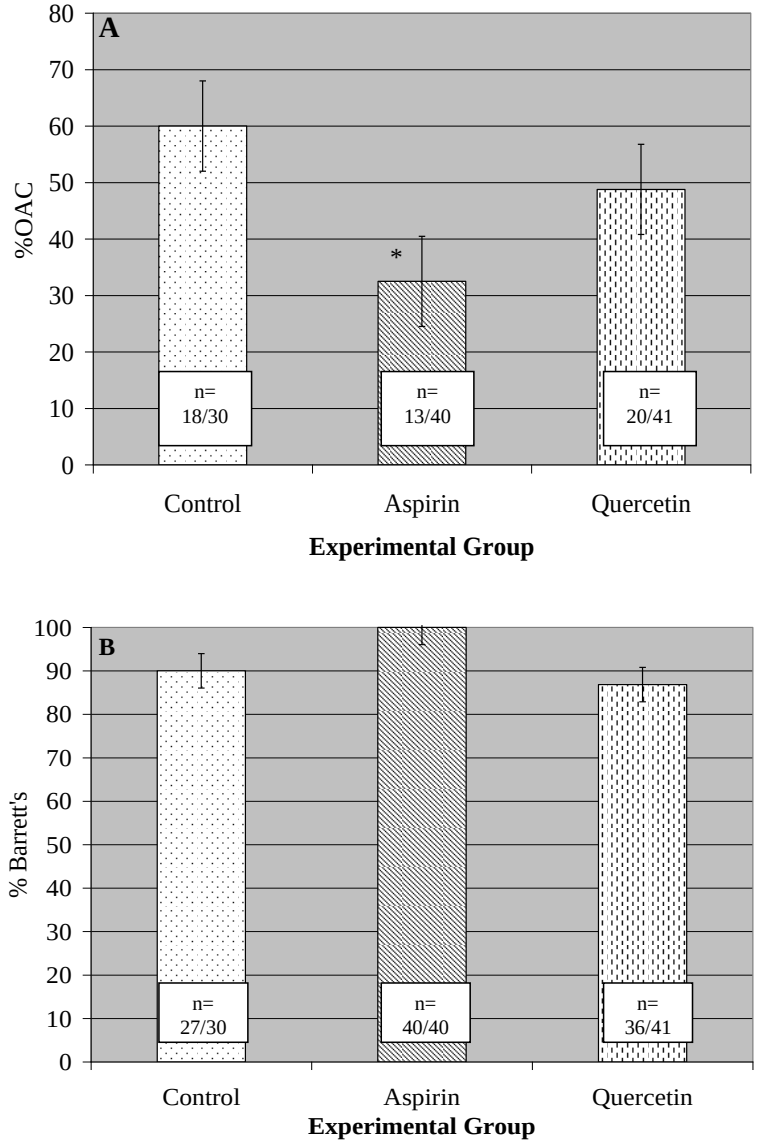

Figure 25. Incidence of OAC and Barrett's oesophagus within the different treatment groups 28 weeks after surgical induction of reflux disease. (**A**) There was a significant decrease in the number of rats with OAC in the aspirin treated group compared to controls (*p=0.003). Fewer cancers were seen in the quercetin treated group compared to controls but the decrease was not statistically significant (p=0.24). (**B**) No difference in the incidence of Barrett's oesophagus was seen in either treatment group compared to controls.

4.2.4 CpG ISLAND METHYLATION

4.2.4i General

Quantitative analysis of CpG island methylation in the *ESR-1*, *p16*, and *HPP1* promoter regions in both Barrett's oesophagus and OAC in both the aspirin and quercetin groups did not show a significant reduction in the level of methylation with either treatment compared to controls. However, there was a trend to reduced levels of methylation at the *ESR-1* allele in Barrett's oesophagus and OAC in both treatment groups. Lower levels of methylation were also seen at the *p16* allele in Barrett's oesophagus in both treatment groups, and at the *HPP1* allele in OAC in the aspirin group.

4.2.4ii *ESR-1*

A small but non-significant reduction in the level of *ESR-1* promoter methylation was seen in both the aspirin (2.08% SEM 0.48) and quercetin groups (1.64% SEM 0.49) in Barrett's oesophagus compared to controls (2.36% SEM 0.35%). A proportionally greater reduction in methylation levels was seen in both the quercetin (1.65% SEM 0.59) and aspirin (2.35% SEM 0.69) groups in OAC compared to controls (3.39% SEM 0.71), but again this reduction did not reach statistical significance for either treatment group. The reduction in methylation levels was most marked in the quercetin group in both tissue types. The levels of methylation were similar within each treatment group in both BO and OAC. The results are illustrated in Figure 26.

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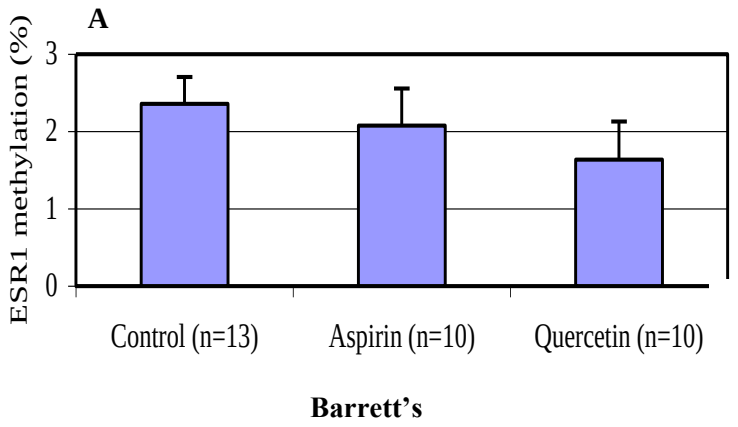

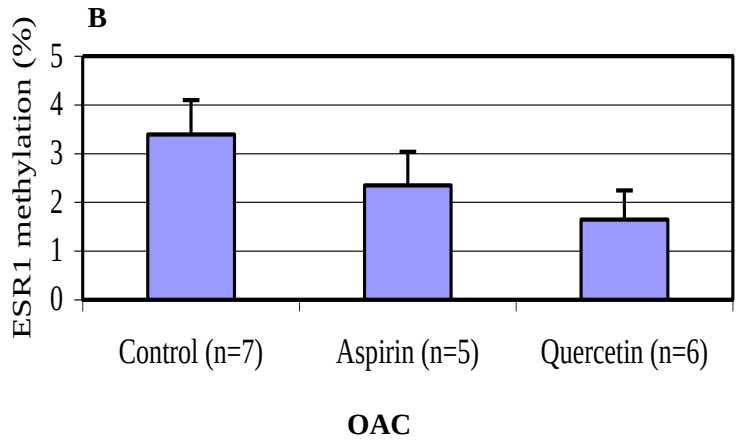

Figure 26. The effect of aspirin and quercetin on the level of CpG island methylation in the *ESR1* promoter region in Barrett's tissue and OAC in rats 28 weeks after surgical induction of reflux disease. Reduced levels of methylation were observed in the aspirin and quercetin treatment groups in Barrett's tissue (**A**) and OAC (**B**) compared to controls, but this reduction did not reach statistical significance. The reduction in degree of methylation was most marked in the quercetin treatment group in both tissue types.

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4.2.4iii *p16*

Lower levels of methylation at the *p16* promoter were present in both the quercetin (0.08% SEM 0.02) and aspirin (0.13% SEM 0.02) groups in Barrett's oesophagus compared to controls (1.68% SEM 1.06), but this reduction did not reach statistical significance. In OAC, methylation levels were similar in both treatment groups (quercetin 0.08% SEM 0.02; aspirin 0.19% SEM 0.09) and controls (0.11% SEM 0.03). There was no discernible difference in the level of methylation within treatment groups between Barrett's oesophagus and OAC, but methylation levels in the control group were an order of magnitude less in OAC compared to Barrett's oesophagus. The results are shown in figure 27.

4.2.4iv *HPP1*

There was no difference in methylation levels of the *HPP1* promoter in Barrett's oesophagus between the quercetin group (0.66% SEM 0.16), or the aspirin group (1.11% SEM 0.52) compared to controls (0.8% SEM 0.17). Lower levels of methylation were present in OAC within all groups compared to Barrett's oesophagus. Methylation levels in OAC in both the aspirin group (0.23% SEM 0.08) and quercetin group (0.39% SEM 0.11) were lower compared to controls (0.59% SEM 0.12). This reduction in methylation level in OAC was most pronounced in the aspirin treated group and at a level approaching statistical significance (p=0.058). The results are shown in Figure 28.

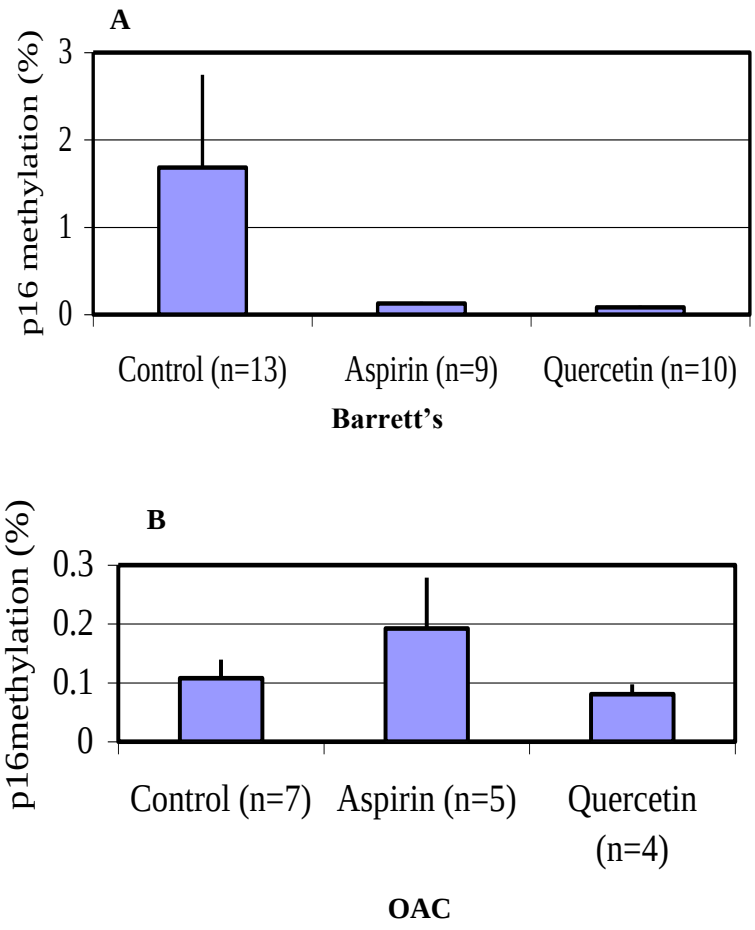

Figure 27. The effect of aspirin and quercetin on the level of CpG island methylation in the *p16* promoter region in Barrett's tissue and OAC in rats 28 weeks after surgical induction of reflux disease. Reduced levels of methylation were observed in the aspirin and quercetin treatment groups in Barrett's tissue (**A**) compared to controls, but this reduction did not reach statistical significance. No discernible difference in methylation levels were seen between any of the groups in OAC (**B**). However, methylation levels in OAC were an order of magnitude less than in Barrett's tissue in the control group.

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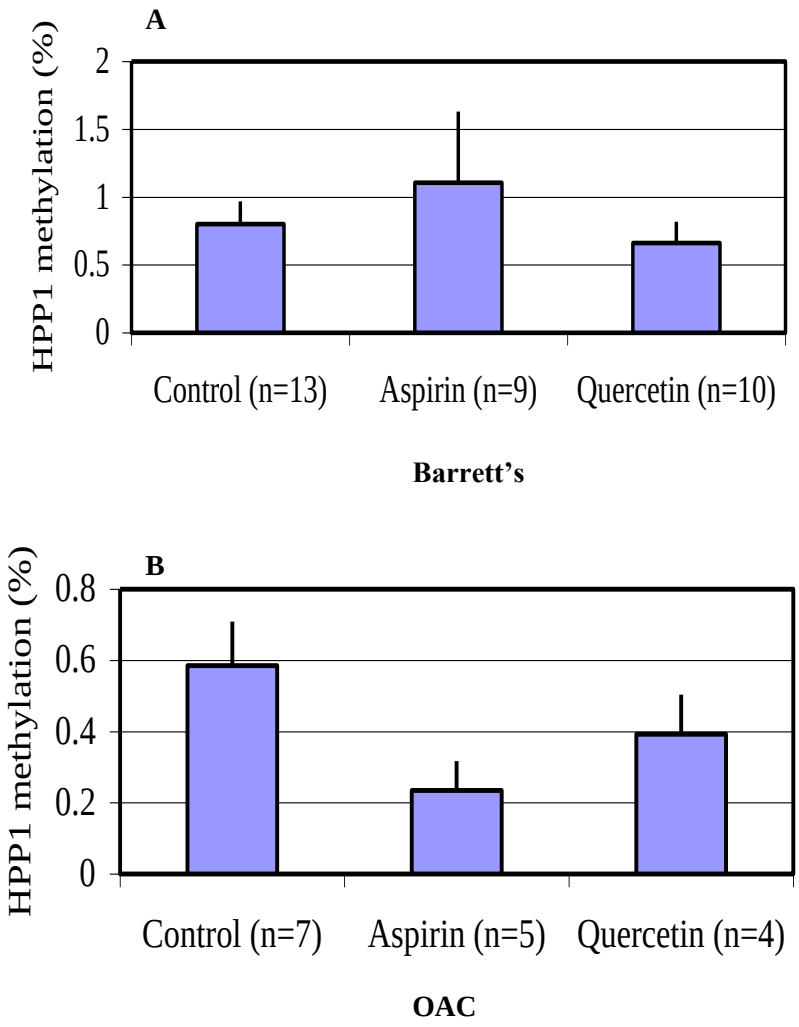

Figure 28. The effect of aspirin and quercetin on the level of CpG island methylation in the *HPP1* promoter region in Barrett's tissue and OAC in rats 28 weeks after surgical induction of reflux disease. The levels of methylation in Barrett's tissue in both the aspirin and quercetin groups were similar to controls (**A**). Reduced levels of methylation were seen in both the aspirin and quercetin group in OAC (**B**) compared to controls. This difference was most marked in the aspirin group where the reduction in methylation approached statistical significance (p=0.058).

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