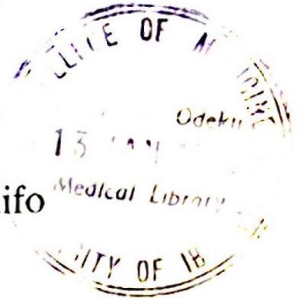


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Effects of gonadectomy on indomethacin-induced ulceration and peptic activity in rats

F. S. OLUWOLE AND A. F. BOLARINWA

Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria

Summary

Castration in rats caused a reduction in the degree of ulceration produced by indomethacin. Compared with the normal intact male rats, the value of the castrated rats was highly significant (18.42 ± 0.22 compared with 9.58 ± 0.17) ($P < 0.01$). However, the normal intact male rats had a greater degree of ulceration than the female rats at the pro-oestrous (2.33 ± 0.13) or oestrous (2.97 ± 0.12) phases. The ovariectomized rats, however, showed no significant reduction ($P > 0.01$) in mean ulcer score when compared with the di-oestrous female rats. The mean value of peptic activity was very high in ovariectomized rats while it was reduced in intact female rats. The ovariectomized rats were more prone to ulceration than intact female rats although the susceptibility increased in rats at di-oestrus.

Résumé

Une des conséquences de la castration des rats est la baisse du degré d'ulcération produit par l'indométhacine. Comparons avec les rats mâles intacts normaux, la valeur de la castration des rats était très significative (18.42 ± 0.22 à 9.58 ± 0.17) ($P < 0.01$). Toutefois les rats mâles intacts normaux, ont un degré plus élevé d'ulcération que chez les rats femelles pré-oestrales (2.33 ± 0.13) ou oestrales (2.92 ± 0.12). Toutefois les rats ayant subi une ovariectomie ne montrent pas de réduction significative ($P < 0.01$) dans le taux de woyeu des ulcères quand on les compare avec des rats femelles di-oestral. La valeur moyenne d'activité peptique était très élevée chez les rats ayant

eu une ovariectomie tandis qu'elle diminuait chez les rats femelles intacts. Les rats ayant eu une ovariectomie avaient plus tendance à développer l'ulcération que chez les rats femelles intacts bien qu'on observe une croissance dans la susceptibilité des rats di-oestrales.

Introduction

Variations in the incidence rates and severity of duodenal ulcers in patients in different parts of the world, most especially India and Africa, have been reported by various workers [1-3]. In childhood, chronic peptic ulcer is uncommon, but when it occurs the two sexes are equally affected [4]. After puberty its frequency increases and a heavy male preponderance becomes apparent [5]. Doll [6] reported that the chance of a man developing a duodenal ulcer was constant between the ages of 20 and 65 yr. By contrast, the chance of a woman developing a duodenal ulcer remains relatively low throughout the whole of her active reproductive life but increases sharply at menopause [7].

Singh *et al.* [8] had reported earlier that increased peptic activity in adult male albino rats was related to increased degree of ulceration. Subsequent to this finding, Alphin *et al.* [9] using pyloric-ligated rats, showed that a gastric perfusion of hydrochloric acid under increasing pressure was necessary to produce gastric ulceration. The degree of gastric ulceration was parallel to increases in intragastric pressure, acidity and peptic activity. However, it has been found that acid and endogenous pepsin secretion are both needed for duodenal ulcerogenesis in rats. This pepsin may be an obligatory ulcerogenic factor in the rat [10].

Using agar gel electrophoresis, pepsin has been identified to occur in five unique forms in

Correspondence: Dr A. F. Bolarinwa, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

man [11]. Of these pepsins of human gastric juice, pepsin 1 has been observed in increased concentration in groups of individuals having an increased risk of acute or chronic ulceration [12]. Pepsin 1 also accounts for an increasing proportion of the total peptic activity per millilitre of gastric juice samples collected under basal conditions from such patients. Patients with duodenal ulcers as a group, have a higher total pepsin secretion than normal subjects or patients with no evidence of recent inflammation [13]. This work was therefore designed to find out the possible association between gonadal hormones and peptic ulceration and peptic activity.

Materials and methods

Male and female adult rats of the Wistar strain were used for the experiments. Bilateral castration and ovariectomy were aseptically performed on some of the rats, using diethyl ether as anaesthetic. The gonadectomized rats were allowed 35 days to recover from the operation and for the wounds to heal completely. Four sets of rats were thus available for use, namely, the intact male, the intact female, the castrated and ovariectomized rats. The female rats were further sub-divided into the oestrous phases, namely, pro-oestrous, oestrous and di-oestrous, by identifying the cells found in their vaginal smears.

Indomethacin is almost insoluble in water, but as it is a carboxylic acid compound (pK_a 4.6), its solubility in water varies with its pH. The drug was therefore suspended in 0.9% normal saline before its administration to the rats. Indomethacin (40 mg/kg) was administered orally using an oro-gastric tube. The induction of ulcer by this method is rapid, only requiring 4 h [14].

The rats were later killed by a blow to the head and their stomachs opened through the lesser curvature and examined macroscopically and microscopically for the presence and scoring of gastric ulceration using the Alphin and Ward method [15].

Examination of the stomach for ulceration and assessment of the degree of ulceration

The stomach was surgically removed and opened up by an incision along the lesser curva-

ture. Macroscopic examination of the stomach was carried out with a hand lens with $\times 2$ magnification. For the microscopic examination of the stomach, histological sections of the stomach were taken and stained with haematoxylin and eosin, and mounted in Canada balsam. Microscopic examination of the section was then carried out under a light microscope.

The 'scoring' technique used for the indomethacin-induced gastric ulceration was assessed with the following criteria:

Criteria	Ulcer score
Normal stomach	0
Punctuate haemorrhage or pin-point ulcers	0.5
Two or more small haemorrhagic ulcers	1
Ulcers greater than 3 mm in diameter	2

A modified method of Sen and Roy [16] for estimating peptic activity in gastric content was used. This method requires a substrate medium for the enzyme pepsin suspected to be present in the gastric contents, hence dehydrated human plasma was used instead of oxycarboxy-haemoglobin.

Results

The results of this investigation indicate that mean values of peptic activity in meq/tyrosine per min were higher in the gonadectomized rats (castrated and ovariectomized) than in the intact male and female rats (Table 1 and Fig. 1). The gonadal influences observed when intact males were compared with castrated males, and intact females compared with ovariectomized females, were highly significant ($P < 0.01$) using Student's *t*-test.

Table 2 shows the summary of results obtained from the mean ulcer score in the following different types of rats, intact male, intact female, castrated and ovariectomized. The castrated rats showed a significant reduction in the degree of ulceration produced by indomethacin than the intact male rats ($P < 0.01$; Fig. 2). The intact male rats also had a greater degree of ulceration than all the female rats in the various oestrous phases ($P < 0.01$)

Table 1. Peptic activity of the gastric contents in different types of rats

Type of rat	Optical density		Peptic activity (meqtyrosine min ⁻¹ at 35.5°C)
	Test	Control	
Intact male (n = 12)	0.68 ± 0.00	0.62 ± 0.00	9.051 ± 0.0347
Castrated (n = 12)	0.71 ± 0.01	0.37 ± 0.00	15.84 ± 0.0612*
Intact female (n = 12)	0.40 ± 0.02	0.30 ± 0.02	11.00 ± 0.0724
Ovariectomized (n = 12)	0.38 ± 0.01	0.18 ± 0.01	17.42 ± 0.1424*

Values of peptic activity are expressed in meqtyrosine min⁻¹ ± s.e., and calculated using the formula described by Sen and Roy [16].

*Significant difference ($P < 0.01$) when compared with the values for the intact male and female rats.

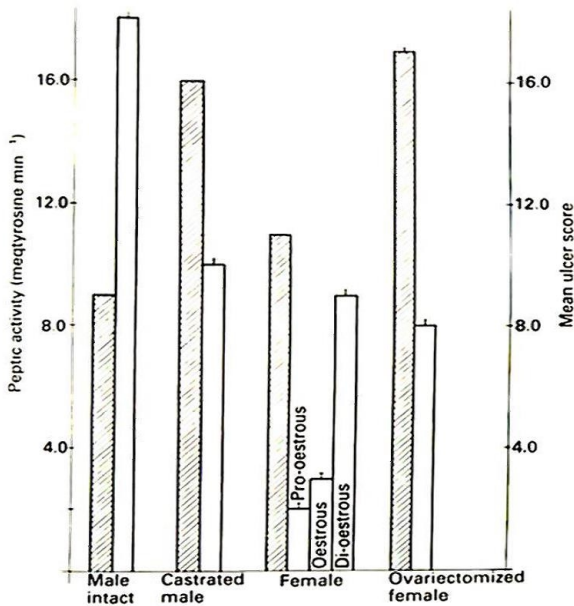


Fig. 1. Mean values of peptic activity and ulcer score.

Discussion

The high values of peptic activity in the ovariectomized rats and the high incidence of gastric ulceration in this group of rats seem to be due to the lack of inhibitory effect that oestrogen has on gastric acid secretion [17]. It is already established that administration of adrenocortical preparation increases the degree and the

incidence of experimentally produced gastric ulceration [18] while natural oestrogen or its synthetic allies significantly inhibit corticosterone secretion [19]. It is equally possible, however, that the increase in the degree of gastric ulceration in ovariectomized animals in the present experiments may be due to the absence of inhibitory influence of the ovarian hormone on the adrenocorticoids. This study also shows that the significantly higher values of peptic activity noted in ovariectomized rats were also related to higher degree of ulceration.

This work shows that the low ulcer scores in the intact female rats are associated with the very low peptic activity in the same group of rats. According to Presl *et al.* [20], oestrogen level is very low in ovariectomized rats while it is high in rats at pro-oestrous.

The finding from this work showed that male castrated rats with low ulcer scores exhibited significantly higher peptic activities. This result suggests that, in addition to elevated pepsin secretion, other factors must also contribute to the lower degree of ulceration in castrated rats than in the intact where there was high ulcer score but low peptic activity. The gastric mucus secreted into the stomach of castrated male rats might be large enough to match the high rates of pepsin secretion. This would therefore provide resistance against pepsin digestion. However, the fact that pepsin produces peptic ulceration has not been disputed. Although no previous work has shown that testosterone inhibits mucus secretion it appears that the decline in the blood testosterone after

Table 2. Summary of results showing mean ulcer score in different types of rats

Groups	Number of animals	Scoring system				Total score	Mean ulcer score \pm s.e.
		0	0.5	1.0	2.0		
Intact male	12	0	21	98	102	221	18.42 \pm 0.22
Castrated	12	0	25	70	20	115	9.58 \pm 0.17
Pro-oestrous female	12	0	6	22	0	28	2.33 \pm 0.13
Oestrous female	12	0	26	9	0	35	2.92 \pm 0.12
Di-oestrous female	12	0	40	34	30	104	8.67 \pm 0.20
Ovariectomized	12	0	27	33	32	92	7.67 \pm 0.16

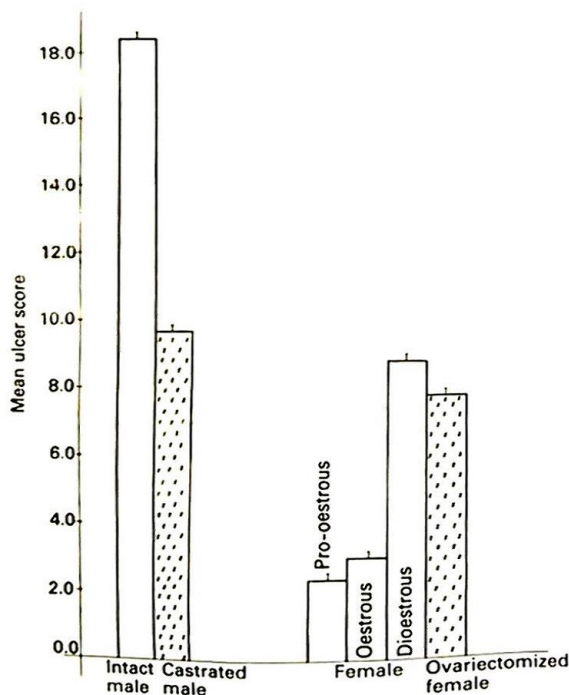


Fig. 2. Effect of gonadectomy on peptic ulceration.

castration might favour gastric mucus secretion in the face of increased pepsin activity.

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